CCN1 expression in hepatocytes contributes to macrophage infiltration in nonalcoholic fatty liver disease in mice

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Abstract  Our objective was to investigate the potential roles of CCN1 in the inflammation and macrophage infiltration of nonalcoholic fatty liver disease (NAFLD). The regulation of hepatic CCN1 expression was investigated in vitro with murine primary hepatocytes treated with free fatty acids or lipopolysaccharide (LPS) and in vivo with high-fat diet-fed mice or ob/ob mice. CCN1 was increased in a diet-specific manner in the liver and induced more severe hepatic inflammation and macrophage infiltration in HF mice than in ND mice. CCN1 recruited macrophages through activation of the NF-κB signaling pathway in myeloid-derived macrophages and RAW264.7 cells. CCN1 expression was also observed in vivo. LPS and FFAs induced CCN1 expression in primary murine hepatocytes in vitro through the TLR4/MyD88/AP-1 pathway. CCN1 protein and overexpression of CCN1 in the liver induced more severe hepatic inflammation and macrophage infiltration in HF mice than in ND mice. CCN1 recruited macrophages through activation of the MeK/Erk signaling pathway in myeloid-derived macrophages and RAW264.7 cells in vitro. Endotoxin and FFA-induced CCN1 expression in hepatocytes is involved in the hepatic proinflammatory response and macrophage infiltration in murine NAFLD.—Bian, Z., Y. Peng, Z. You, Q. Wang, Q. Miao, Y. Liu, X. Han, D. Qiu, Z. Li, and X. Ma. J. Lipid Res. 2013. 54: 44–54.

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Nonalcoholic fatty liver disease (NAFLD) begins with the aberrant accumulation of triglycerides in the liver (simple steatosis), which in some individuals elicits an inflammatory response (nonalcoholic steatohepatitis, NASH) that can progress to cirrhosis and liver cancer (1). NASH is characterized by hepatocellular ballooning, lobular inflammation, and fibrosis in histology (2–4). A good model to explain the pathogenesis of NASH is the “multiple parallel hits” hypothesis proposed by Tilg and Moschen, which states that various parallel processes, especially gut-derived and adipose tissue-derived factors, may be conducive to the development of liver inflammation in NAFLD (5).

Members of the CCN (Cyr61/CTGF/NOV) family have emerged as dynamically expressed, extracellular matrix-associated proteins that play critical roles in regulating cell adhesion, migration, proliferation, differentiation, apoptosis, and survival (6, 7). CCN1 (also known as cysteine-rich protein 61, Cyr61) is normally expressed at a low level in most tissues but is increased as a result of inflammation or tissue repair. It has been shown that CCN1 activates the NF-κB signaling pathway in macrophages, leading to the expression of multiple proinflammatory cytokines and chemokines characteristic of classical activated M1 macrophages (8). The definitive role of CCN1 in liver diseases remains unclear, although it has been shown to be a tumor suppressor in hepatocarcinogenesis (9) and is involved in the oxidative DNA damage response in ConA-treated livers (10). In this study, we showed for the first time that induction of CCN1 by
FFAs and LPS in hepatocytes resulted in macrophage infiltration and inflammation in the liver in hepatic steatosis.

MATERIALS AND METHODS

Animal and treatments

Male C57BL/6 mice (6–8 weeks of age) were purchased from the Shanghai SLAC Laboratory Animal Co. Ltd (Shanghai, China) and housed under pathogen-free conditions in the animal facility of the Shanghai Jiao Tong University School of Medicine. Mice were fed either a high-fat (HF) diet or an isocaloric normal diet (ND) containing less fat (12% fat, 59% total carbohydrate, and 29% protein). Male C57BL/6 ob/ob mice (6–8 weeks of age) were obtained from the Model Animal Research Center of Nanjing University (Nanjing, China). All mice were maintained in a temperature- and light-controlled facility with ad libitum access to food and water.

Escherichia coli LPS (50 µg/mouse, Sigma, St. Louis, MO) was injected intraperitoneally, and mice were euthanized 6 h later. The CCN1 protein displays a remarkable degree of evolutionary conservation, with 92.8% identity between mouse and human CCN1 (11). Recombinant human CCN1 (5 µg/mouse, R and D Systems, Minneapolis, MN) was administered intravenously through the tail vein, and mice were euthanized 24 h later. The neutralizing anti-integrin α4 antibody (0.1 mg/mouse, BD Pharmingen, San Diego, CA) or Z-VAD-FMK (200 µg/mouse, Sigma) which inhibits induction of apoptosis, was injected through the tail vein 2 h before administration of CCN1. Control animals were injected with vehicle only.

Liver-specific CCN1 expression plasmid construction

The construction of the liver-specific CCN1 expression plasmid was carried out as previously described (12), using the Enh1mTTR (ET) promoter generated by fusing a synthetic hepatocyte-specific enhancer to the murine transthyretin promoter (13). The CCN1 cDNA inserted into the vector was expressed under the control of ET promoter. The control plasmid contained the GFP gene under control of the same promoter.

Cell culture

Primary murine hepatocytes were isolated from mice by in situ collagenase liver perfusion (14) and cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 100 µg/ml streptomycin, and 100 U/ml penicillin (Invitrogen, Carlsbad, CA).

Primary murine macrophages were obtained as previously described with minor modifications (15) and cultured in DMEM supplemented with 10% FBS, 20 ng/ml macrophage colony-stimulating factor (M-CSF; R and D Systems), 100 µg/ml streptomycin, and 100 U/ml penicillin. The macrophage RAW264.7 cell line was purchased from the Shanghai Institute of Cell Biology (Chinese Academy of Sciences, Shanghai, China) and cultured with DMEM plus 10% FBS.

Cells were precultured with JNK inhibitor II (SP600125, 10 µM), IKK-2 inhibitor V (IMD0354, 1 µM), U0126 (10 µM), PD98059 (10 µM) (Merck, Darmstadt, Germany), or anti-integrin α4 (5 µg/ml, BD Pharmingen) for 2 h before stimulation with LPS, OP 2:1 (mixture of oleic acid and palmitic acid at a ratio of 2:1, Sigma) or CCN1, respectively.

Chemotaxis assay

RAW264.7 cells or murine myeloid-derived macrophages were precultured with or without neutralizing anti-integrin α4 (5 µg/ml, U0126 (10 µM) for 30 min, and then 1 × 10^6 cells were placed on a 0.8 µm membrane (Millicell H anger Cell Culture Insert, PET, Millipore, Billerica, MA) in DMEM with 10% FBS. The cells were allowed to migrate toward CCN1 in the medium below the membrane at the indicated concentrations (250 ng/ml, 500 ng/ml, 1,000 ng/ml) for 90 min and 24 h, respectively. Migration was quantified as the number of crystal violet-stained cells observed on the underside of the membrane by light microscopy (10 randomly chosen fields per membrane and three replicate wells per treatment).

Western blot

Immunoblotting analyses were carried out using either cell extracts or whole-liver extracts, as described previously (16). Immunoblots were probed with antibodies to CCN1 (Abcam, Cambridge, UK), phosphorylated and total Mek1/2, Erk1/2, C-jun, and IkB-α (Cell Signaling Technology, Boston, MA).

Immunohistochemistry and TUNEL assay

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded liver tissues using antibodies against CD11b (Abcam, Cambridge, UK), phosphorylated and total Mek1/2, Erk1/2, C-jun, and IkB-α according to the manufacturer’s instructions.

Statistical analysis

Data are expressed as the mean ± SD. The group means were compared using ANOVA. All statistical analysis was performed using the SPSS statistical software version 16.0 (SPSS Inc., Chicago, IL). P values < 0.05 were considered statistically significant.

RESULTS

LPS increases hepatic CCN1 expression in murine NAFLD

Our previous studies demonstrated that mice fed HF diets became obese and developed hepatic steatosis (18, 19). In the present study, HF mice also developed significant hepatic steatosis characterized by the presence of macrovesicular and microvesicular lipid droplets. Immunohistochemical analysis showed that LPS administration significantly increased hepatic CCN1 expression in HF mice compared with HF mice without LPS administration and LPS-treated ND mice (Fig. 1A, upper panel). To confirm that hepatic CCN1 was increased in different steatosis models, ob/ob mice were treated with or without LPS. Similarly, hepatic CCN1 expression was increased in ob/ob mice compared with WT mice. LPS treatment further increased hepatic CCN1 expression in ob/ob mice (Fig. 1A, lower panel).
in the liver is hepatocytes (Fig. 1D). Therefore, we chose hepatocytes as target cells for our next experiments to explore the regulation of CCN1 expression.

**LPS and FFAs induce CCN1 expression in primary murine hepatocytes through the TLR4/MyD88/AP-1 signaling pathway**

LPS at the concentrations of 1 µg/ml and 2 µg/ml induced CCN1 expression by approximately 3-fold in primary murine hepatocytes in vitro (Fig. 2A, upper panel). In addition, CCN1 expression was detectable at
12 h and reached a maximum value at 48 h after LPS treatment at 2 µg/ml (Fig. 2A, lower panel). Primary murine hepatocytes were incubated with a mixture of long chain fatty acids (OP 2:1) to establish a cellular steatosis model (20). Similarly, FFAs (OP 2:1) also induced CCN1 expression in a concentration-dependent manner, peaking at 0.5 mmol/l (Fig. 2B, upper panel). At 0.5 mmol/l, FFAs were shown to induce the maximum CCN1 expression level at 24 h (Fig. 2B, lower panel). These results demonstrated that LPS and FFAs both induced CCN1 expression of hepatocytes in a time- and concentration-dependent manner.

In vivo, LPS administration nearly tripled the hepatic CCN1 expression in WT mice; however, no significant difference in CCN1 expression was detected between treatment with LPS and treatment without LPS in TLR4−/− and MyD88−/− mice (Fig. 3A). Similarly, LPS and FFAs could not induce CCN1 expression in hepatocytes isolated from TLR4−/− or MyD88−/− mice (Fig. 3B). These results suggest that LPS and FFA-induced CCN1 expression is dependent on the TLR4/MyD88 signaling pathway.

To illustrate the downstream signaling pathway of TLR4/MyD88 involved in this process, inhibitors for the AP-1 (SP600125) and NF-κB (IMD0354) pathways were used. SP600125 and IMD0354 significantly inhibited the phosphorylations of c-Jun (component of AP-1) and IκBα, respectively (supplementary Fig. I-A). However, only SP600125 inhibited the expression of CCN1 in primary hepatocytes stimulated with LPS or FFAs (Fig. 3C). These results suggest that LPS and FFAs induce CCN1 expression in hepatocytes through activation of the TLR4/MyD88/AP-1 signaling pathway.

Fig. 2. LPS and FFAs induce CCN1 expression in murine primary hepatocytes in vitro. (A) Representative autoradiographs of CCN1 in primary hepatocytes treated with LPS at the indicated dosages and time points. (B) CCN1 expression in murine primary hepatocytes treated with OP 2:1 at the indicated dosages and time points. (n = 3 in each group, *P < 0.05; **P < 0.01).
CCN1 induces severe hepatic inflammation and liver injury in murine NAFLD

CCN1 protein induced mild to moderate hepatic inflammation in ND mice, whereas it induced severe hepatic inflammation in HF mice (Fig. 4A). CCN1 treatment increased the numbers of hepatic inflammatory foci in both ND and HF mice compared with ND and HF mice without treatment, respectively. Furthermore, CCN1 induced...
Fig. 4. CCN1 induces severe hepatic inflammation in murine fatty liver. (A) Representative H and E staining of liver sections (magnification 200×) from ND and HF mice that were either untreated or treated with CCN1 protein with or without neutralizing antibody against integrin αvβ3. (B) Inflammatory foci per 20 × 10 field in liver tissues obtained from ND and HF mice that were either untreated or treated with CCN1 protein with or without neutralizing antibody against integrin αvβ3. (C) Mean (±SD) serum ALT levels of ND and HF
more hepatic inflammatory foci in HF mice compared with ND mice treated with CCN1 (Fig. 4B). Similarly, CCN1 treatment increased serum ALT and AST levels in both ND and HF mice (Fig. 4C, D). Consistent with the histological changes, CCN1-treated HF mice had higher ALT levels than CCN1-treated ND mice (Fig. 4C), suggesting that the HF mice were more susceptible to CCN1-induced hepatic inflammation and liver injury.

As CCN1 induces macrophage adhesion and activation through integrin αMβ2 (8, 21), a neutralizing antibody against integrin αM was used to block the binding between CCN1 and macrophages. Neutralization of integrin αM attenuated CCN1-induced hepatic inflammation in ND and HF mice, indicated by less hepatic inflammatory infiltration in histological examinations and lower serum ALT and AST levels compared with ND and HF mice treated with CCN1 only (Fig. 4A–D). These results suggested that CCN1 induced monocyte infiltration through binding to integrin αM on the surface of monocytes. CCN1 also induced moderate hepatic inflammation in ob/ob mice but not to the extent seen in HF mice (supplementary Fig. 1-B). Delivery of a liver-specific CCN1 plasmid, confirmed to successfully overexpress CCN1 in ND and HF mice (supplementary Fig. 1-B). CCN1-induced liver injury. More importantly, CCN1 induced more hepatic inflammatory foci in both ND and HF mice (Fig. 4E).

**CCN1 induces macrophage infiltration through binding to integrin αM in vivo**

Next, we analyzed macrophage infiltration in CCN1 protein-induced hepatic inflammatory foci in both ND and HF mice by using F4/80 immunohistochemistry. F4/80 positive cells were frequently detected (Fig. 5A), suggesting that macrophages are the major cellular type infiltrated in CCN1-induced liver injury. More importantly, CCN1 induced more macrophage infiltrates in HF mice compared with ND mice treated with CCN1 (Fig. 5B). According to the results of integrin αM decreased CCN1-induced hepatic macrophage infiltrates in ND and HF mice (Fig. 5A, B). Flow cytometric analysis showed that the majority of F4/80 positive cells in the liver expressed integrin αM (Fig. 5C). These results suggest that integrin αM may mediate macrophage infiltration in CCN1-induced hepatic inflammation. Consistent with the results of CCN1 protein administration, the forced expression of CCN1 also induced numerous macrophage infiltrations in both ND and HF mice (Fig. 5D).

**CCN1-induced hepatic inflammation and macrophage infiltration are not affected by apoptosis inhibitor in vivo**

It was reported that CCN1 could facilitate apoptosis mediated by Fas and TNF-α in vivo and vitro (10, 22). Therefore, we observed whether cellular apoptosis played a critical role in hepatic inflammation and macrophage infiltration induced by CCN1. Indeed, CCN1 induced cellular apoptosis in the livers not only of ND mice but also of HF mice, and Z-VAD-FMK, a caspase inhibitor, restrained this process (Fig. 6A). However, Z-VAD-FMK did not significantly attenuate the hepatic inflammation induced by CCN1 in either ND or HF mice (Fig. 6B). In addition, CCN1-induced macrophage infiltration was not significantly reduced by Z-VAD-FMK (Fig. 6C), indicating that the role of CCN1 on hepatic inflammation and macrophage infiltration is independent of cellular apoptosis potentially induced by CCN1.

**CCN1 induces macrophage chemotaxis through the integrin αM/Mek1/2/Erk1/2 signaling pathway in vitro**

CCN1 in different dosages increased the phosphorylation of Mek1/2 and Erk1/2 in the RAW264.7 cell line (Fig. 7A). CCN1 recruited primary murine myeloid-derived macrophages in a concentration-dependent manner, ranging from 250 to 1,000 ng/ml both at 90 min and 24 h. Pretreatment with the neutralizing antibody against integrin αM or an inhibitor for Mek1/2 (U0126) significantly reduced the level of macrophage migration in response to CCN1 (Fig. 7B). Similar results were also found in the murine macrophage RAW264.7 cell line (Fig. 7C). Taken together, these results suggest that CCN1 recruits macrophages through activation of the integrin αM/Mek1/2/Erk1/2 signaling pathway.

**DISCUSSION**

In this study, we demonstrated for the first time that FFAs and LPS induced CCN1 expression in hepatocytes through the TLR4/MyD88/AP-1 signaling pathway in vitro and in vivo. CCN1 could induce severe hepatic inflammation and apoptosis in mice with NAFLD, and CCN1 was shown to induce macrophage chemotaxis through the integrin αM/Mek1/2/Erk1/2 signaling pathway. Therefore, CCN1 may play roles in the inflammatory response and macrophage infiltration in NAFLD.

Accumulating evidence shows that innate immunity is actively involved in insulin resistance and plays a critical role in the pathogenesis of NASH (23). TLR4 links innate immunity and fatty acid-induced insulin resistance (24, 25). TLR4 is a high-affinity receptor for LPS, a component of the cellular wall of gram-negative bacteria, and is considered a pivotal exogenous danger-signaling molecule in the pathogenesis of NAFLD (26). LPS has been shown to induce production of TNF-α in macrophages, thereby triggering TNF-α-induced hepatocyte apoptosis in a murine NASH model (27). In addition, TLR4 plays an important role in mediating proinflammatory effects of saturated FFAs. Saturated FFAs may function as the endogenous substances that contribute to TLR4 activation in the setting of obesity (28). FFAs

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mice that were either untreated or treated with CCN1 protein with or without neutralizing antibody against integrin αM. (D) Mean (±SD) serum AST levels of ND and HF mice that were either untreated or treated with CCN1 protein with or without neutralizing antibody against integrin αM. (E) Representative H and E staining of liver sections (magnification 200×) from ND and HF mice with forced CCN1 expression in the liver. (n = 5 in each group, *P < 0.05, **P < 0.01).
may elicit hepatotoxicity and stimulate progression from simple steatosis to NASH via several mechanisms beyond direct cytotoxicity (29). Palmitic acid can bind to TLR4, leading to activation of NF-κB and upregulate its target genes, such as TNF-α and IL-6, in macrophages and adipocytes. TLR4-deficient mice develop obesity when fed a HF diet; however, they are still partially protected against development of insulin resistance, possibly due to reduced inflammatory gene expression in liver and fat tissues (24). FFAs also induce production of proinflammatory cytokines TNF-α (30) and IL-8 (31) from hepatocytes, potentially contributing to hepatic inflammation and consequent liver injury. Most recently, Csak and colleagues showed that the saturated FFA palmitic acid upregulates the inflammasome, which cleaves pro-interleukin-1β (pro-IL-1β) into secreted IL-1β, and induces sensitization to LPS for IL-1β release in hepatocytes (32). Our study showed that LPS and FFAs induced production of CCN1 in hepatocytes, which may be another important inflammatory factor in the liver. Therefore, our study and other studies suggest that hepatocytes are not only passively injured targets but that they also are actively involved in orchestrating responses to insults in NASH.

Fig. 5. CCN1 induces macrophage infiltration in murine NAFLD. (A) Representative immunohistochemical stain of F4/80 in the livers (magnification 400×) of ND and HF mice that were either untreated or treated with CCN1 protein with or without neutralizing antibody against integrin α5. (B) Numbers of F4/80 positive cells per 40 × 10 field in liver tissues obtained from mice that were either untreated or treated with CCN1 protein with or without neutralizing antibody against integrin α5. (C) Flow cytometric analysis of F4/80 and integrin α5 expression in monocytes (gated on CD45 positive cells) from murine livers. (D) Representative immunohistochemical stain of F4/80 in liver sections (magnification 200×) from ND and HF mice with forced CCN1 expression. (n = 5 in each group, *P < 0.05, **P < 0.01).
Macrophages are another important component of innate immunity in NAFLD. In adipose tissue, macrophages are a major source of proinflammatory cytokines, which can function in a paracrine and potentially an endocrine fashion to cause decreased insulin sensitivity. The accumulating evidence indicates that activation of hepatic macrophages (Kupffer cells) positioned at the “frontline” is an essential element in the pathogenesis of NAFLD (25). It was shown that CCN1 can activate a proinflammatory genetic program in macrophages, such as monocyte chemotactic protein 1 (MCP-1) and macrophage inflammatory protein 1 α (MIP-1α) (8). In our study, we showed...
Fig. 7. CCN1 recruits macrophages through the integrin α5/Mek1/2/Erk1/2 signaling pathway. (A) Representative autoradiographs of Mek1/2 and Erk1/2 activation in RAW264.7 cells stimulated with CCN1 for 30 min in the indicated dosages. (B) Migration of murine myeloid-derived macrophages toward indicated concentrations of CCN1 for 90 min and 24 h, and precultured with or without neutralizing anti-integrin α5 or U0126. (C) Effect of CCN1 on macrophage chemotaxis was assessed in the murine macrophage RAW264.7 cell line. These experiments were repeated three times. (*P < 0.05, **P < 0.01).
that CCN1 could recruit macrophages through binding to integrin αMβ2 on the cellular surface of macrophages, which at least partially explained the massive macrophage infiltrates in murine liver after CCN1 administration. Therefore, production of CCN1 by hepatocytes after treatment with LPS and FFAs may activate and recruit macrophages into the liver. These activated hepatic macrophages then release various chemokines, such as MCP-1, which in turn recruit additional macrophages, setting up a feed-forward process that further increases the number of macrophages in the liver and propagates the chronic inflammatory state (28). In other words, the proinflammatory process is initiated in NALFD by CCN1-mediated induction of macrophage infiltration into the liver through direct chemotaxis and secondary recruitment by chemokines.

In summary, we propose for the first time that endotoxins and FFAs induce expression of the matricellular signaling molecule CCN1 in hepatocytes through activation of the TLR4/MyD88/AP-1 signaling pathway in mice. CCN1 then drives the recruitment of macrophages into the steatotic liver, inducing the inflammatory process and macrophage infiltration. The potential role of CCN1 in the process of transition from simple steatosis to NASH in humans will be worth exploring in future studies. Thus, CCN1 may become a potential therapeutic target to prevent disease progression to advanced stages in NALFD.

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REFERENCES

1. Cohen, J. C., J. D. Horton, and H. H. Hobbs. 2011. Human fatty liver disease: old questions and new insights. Science. 332: 1519–1523.

2. Farrell, G. C., and C. Z. Larter. 2006. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology. 43: S99–S112.

3. Kleiner, D. E., E. M. Brunt, M. Van Natta, C. Behling, M. J. Contos, O. W. Cummings, L. D. Ferrell, Y. C. Liu, M. S. Torbenson, A. Unalp-Arida, et al. 2005. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 41: 1313–1321.

4. Brunt, E. M. 2004. Nonalcoholic steatohepatitis. Semin. Liver Dis. 24: 3–20.

5. Tilg, H., and A. R. Moschen. 2010. Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. Hepatology. 52: 1836–1846.

6. Chen, C. C., and L. F. Lau. 2009. Functions and mechanisms of action of CCN matricellular proteins. Int. J. Biochem. Cell Biol. 41: 771–783.

7. Perbal, B. 2004. CCN proteins: multifunctional signalling regulators. Lancet. 363: 62–64.

8. Bai, T., C. C. Chen, and L. F. Lau. 2010. Matricellular protein CCN1 activates a proinflammatory genetic program in murine macrophages. J. Immunol. 184: 5228–5232.

9. Feng, P., B. Wang, and E. C. Ren. 2008. Cyr61/CCN1 is a tumor suppressor in human hepatocarcinoma carcinoma and involved in DNA damage response. Int. J. Biochem. Cell Biol. 40: 98–109.

10. Chen, C. C., J. L. Young, R. I. Monzon, N. Chen, V. Todorovic, and L. F. Lau. 2007. Cytotoxicity of TNFα mediated by integrin-mediated matrix signaling. EMBO J. 26: 1257–1267.

11. Jay, P., J. L. Berge-Lefranc, G. Marsollier, C. Mejean, S. Tavavia, and P. Berta. 1997. The human growth factor-inducible immediate-early gene, CYR61, maps to chromosome 1p. Oncogene. 16: 1753–1757.

12. Le Saux, O., K. Fulop, Y. Yamaguchi, A. Ilias, Z. Szabo, C. N. Brampton, V. Pomozi, K. Huszár, T. Arányi, and A. Váradi. 2011. Expression and in vivo rescue of human ABCG5 disease-causing mutations in mouse liver. PLoS ONE. 6: e19478.

13. Mártaí, J., A. Cantore, C. C. Bartholomae, A. Annoni, W. Wang, A. Acosta-Sánchez, E. Samara-Kuku, L. De Waele, L. Ma, P. Genovesi, et al. 2011. Hepatocyte-targeted expression by integrase-defective lentiviral vectors induces antigen-specific tolerance in mice with low genotoxic risk. Hepatology. 53: 1696–1707.

14. Nyagode, B. A., C. M. Lee, and E. T. Morgan. 2010. Modulation of hepatic cytochrome P450s by Citrobacter rodentium infection in interleukin-6 and interferon-γnull mice. J. Pharmacol. Exp. Ther. 335: 480–488.

15. Gersuk, G. M., L. W. Razai, and K. A. Marr. 2008. Methods of in vitro macrophage maturation confer variable inflammatory responses in association with altered expression of cell surface decoy-1. J. Immunol. Methods. 329: 157–166.

16. Ma, X., J. Hua, and Z. Li. 2008. Probiotics improve high fat diet-induced hepatic steatosis and insulin resistance by increasing hepatic NKT cells. J. Hepatol. 49: 821–830.

17. Birjandi, S. Z., J. A. Ippolito, A. K. Ramadorai, and P. L. Witte. 2011. Alterations in marginal zone macrophages and marginal zone B cells in old mice. J. Immunol. 186: 4414–4421.

18. Li, Z. J., M. J. Goloski, and A. M. Diehl. 2005. Dietary factors alter hepatic innate immune system in mice with nonalcoholic fatty liver disease. Hepatology. 42: 880–885.

19. Ma, X., J. Hua, A. R. Mohamood, A. R. Hamad, R. Ravi, and Z. Li. 2007. A high-fat diet and regulatory T cells influence susceptibility to endotoxin-induced liver injury. Hepatology. 46: 1519–1529.

20. Feuer, M. A., A. A. Canbay, M. E. Guicciardi, H. Higuchi, S. F. Bronk, and G. J. Gores. 2003. Diet associated hepatic steatosis sensitizes to Fas-mediated liver injury in mice. J. Hepatol. 39: 978–983.

21. Schober, J. M., N. Chen, T. M. Grzeskiewicz, I. Jovanovic, E. E. Emeson, T. P. Ugarova, R. D. Ye, L. F. Lau, and S. C. Lam. 2002. Identification of integrin alphaM/beta2(2) as an adhesion receptor on peripheral blood monocytes for Cyr61 (CCN1) and connective tissue growth factor (CCN2): immediate-early gene products expressed in atherosclerotic lesions. Blood. 99: 4457–4465.

22. Juric, V., C. C. Chen, and L. F. Lau. 2009. Fas-mediated apoptosis is regulated by the extracellular matrix protein CCN1 (CYR61) in vitro and in vivo. Mol. Cell. Biol. 29: 3266–3279.

23. Maher, J. J., P. Leon, and J. C. Ryan. 2008. Beyond insulin resistance: innate immunity in nonalcoholic steatohepatitis. Hepatology. 48: 670–678.

24. Shi, H., M. V. Kokoeva, K. Inouye, I. Tzameli, H. Yin, and J. S. Flier. 2006. TLR4 links innate immunity and fatty acid-induced insulin resistance. J. Clin. Invest. 116: 3017–3025.

25. Baffy, G. 2009. kupffer cells in non-alcoholic fatty liver disease: the emerging view. J. Hepatol. 51: 212–223.

26. Poltorak, A. X., H. I. Smirnova, M. Y. Liu, C. Van Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, et al. 1998. Defective LPS signaling in C3H/HeJ and C3H/HeN mice: mutations in Tlr4 gene. Science. 282: 2085–2088.

27. Kudo, H., T. Takahara, Y. Yata, K. Kawai, W. Zhang, and T. Sugiyama. 2009. Lipopolysaccharide triggered TNF-alpha-induced hepatocyte apoptosis in a murine non-alcoholic steatohepatitis model. J. Hepatol. 51: 168–175.

28. Olefsky, J. M., and C. K. Glass. 2010. Macrophages, inflammation, and insulin resistance. Annu. Rev. Physiol. 72: 219–246.

29. Jou, J., S. S. Choi, and A. M. Diehl. 2008. Mechanisms of disease progression in nonalcoholic fatty liver disease. Semin. Liver Dis. 28: 370–379.

30. Feldstein, A. E., N. W. Wernerburg, A. Canbay, M. E. Guicciardi, S. F. Bronk, R. Rydzewski, L. J. Burgart, and G. J. Gores. 2004. Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression via a lysosomal pathway. Hepatology. 40: 183–194.

31. Yoshi-Barve, S., S. S. Barve, K. Amancherla, L. Gobejishvili, D. Hill, M. Cave, P. Hote, and C. J. McClain. 2007. Palmitic acid induces production of proinflammatory cytokine interleukin-8 from hepatocytes. Hepatology. 46: 823–830.

32. Cuk, T., M. Ganz, J. Pespata, K. Kodys, A. Dolganiuc, and G. Szabo. 2011. Fatty acid and endotoxin activate infl ammasomes in mouse hepatocytes that release danger signals to stimulate immune cells. Hepatology. 54: 133–144.

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