Animal Models Correlating Immune Cells for the Development of NAFLD/NASH

Srikanth Iyer, Pramod Kumar Upadhyay, Subeer S. Majumdar, Perumal Nagarajan

National Institute of Immunology, New Delhi 110067, India

This review mainly elaborates on the animal models available for understanding the pathogenesis of the second hit of non-alcoholic fatty liver disease (NAFLD) involving immune system. This is known to be a step forward from simple steatosis caused during the first hit, which leads to the stage of inflammation followed by more serious liver conditions like non-alcoholic steatohepatitis (NASH) and cirrhosis. Immune-deficient animal models serve as an important tool for understanding the role of a specific cell type or a cytokine in the progression of NAFLD. These animal models can be used in combination with the already available animal models of NAFLD, including dietary models, as well as genetically modified mouse models. Advancements in molecular biological techniques enabled researchers to produce several new animal models for the study of NAFLD, including knockin, generalized knockout, and tissue-specific knockout mice. Development of NASH/NAFLD in various animal models having compromised immune system is discussed in this review. (J Clin Exp Hepatol 2015;5:239–245)

Non-alcoholic fatty liver disease (NAFLD) is a group of syndromes ranging from hepatic steatosis to more severe forms, including non-alcoholic steatohepatitis (NASH) and cirrhosis, which may further progress to hepatocellular carcinoma (HCC) in some cases.[1] Hepatic steatosis is the simple accumulation of fats in the liver cells without any inflammation. The condition of NASH, first described by Ludwig et al. in 1980,[2] involves inflammation of the hepatocytes with lipid accumulation. NAFLD is the most common cause of chronic liver disease.[3] About 20% of the worldwide adult population presents symptoms of simple steatosis, whereas 3% of the adult population is reported to be suffering from NASH.[4,5] NAFLD is frequently associated with other complications as insulin resistance, obesity, and dyslipidemia.[6] According to some estimates, 70–80% of the obese patients suffer from NAFLD[7] while 88% of NASH patients present insulin resistance.[8] Though the precise mechanism of NAFLD is still unknown, its pathogenesis is often described by a two-hit hypothesis: the first involves accumulation of lipids within the hepatocytes (hepatic steatosis), thereby sensitizing the immune cells; the second hit involves the inflammation of liver, leading to NASH and other severe manifestations.

Liver is an important site for the innate component of the immune system in the body. It has a wide variety of immune cells like Kupffer macrophages, lymphocytes, natural killer (NK) cells, and NKT cells. Liver also synthesizes a variety of proteins involved in innate immunity like acute-phase proteins (APPs), complement factors, and pattern recognition receptors (PRRs).

Numerous animal models, particularly mouse models, have been developed for the study of NAFLD/NASH. An ideal animal model is the one that manifest similarity in pathogenesis of NAFLD to that of humans. The animal models can be broadly placed in three categories: dietary models, genetic models, and combination models.

MOUSE KNOCKOUT MODELS FOR NAFLD

T Cell-deficient Models

Hepatic steatosis is responsible for sensitizing the immune cells, particularly the intra-hepatic $CD8^+$ T cells, which on activation leads to hepatic inflammation (NASH) in co-operation with the intra-hepatic NKT cells.[9] $CD8^+$ T cells are one of the earliest cells to get sensitized and activated by lipid accumulation. Nishimura et al.[10] suggested the infiltration of these cells in the adipose tissue of mice fed with high-fat diet, even before the recruitment of macrophages. Depletion of $CD8^+$ T cells reduces inflammation and minimizes macrophage infiltration within the obese adipose tissue. We have previously shown the role of $CD8^+$ T cells in the initiation and propagation of inflammation in the liver, as well as insulin resistance after a high-fructose diet, consequently leading to hepatosteatosis.[11] Mice models deficient in $CD8^+$ T cells include $TAP1^{-/-}$ mice. $TAP1^{-/-}$ mice lack the ability to load MHC class I antigens onto the cell surface, thereby resulting in the failure of generation of

Keywords: mouse models, immune cells, NAFLD
Received: 20.04.2015; *Accepted:* 8.06.2015; *Available online:* 9 July 2015
Address for correspondence: Perumal Nagarajan, National Institute of Immunology, Experimental Animal Facility, JNU Campus, New Delhi 110067, India. Tel.: +91 11 26703709; fax: +91 11 26742125.
E-mails: nagarajan@nii.ac.in, naga73@yahoo.com
Abbreviations: APPs: acute-phase proteins; BAFF: B cell activating factor; Btk: Bruton's tyrosine kinase gene; DAMPs: damage-associated molecular patterns; HCC: hepatocellular carcinoma; IRFs: Interferon regulatory factors; JNK: c-Jun N-terminal kinase; MCD: methionine choline-deficient; NAFLD: non-alcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; NLRs: Nod-like receptors; PAMPs: pathogen-associated molecular patterns
http://dx.doi.org/10.1016/j.jceh.2015.06.004

$CD8^+$ T cells. These mice are protected against NAFLD and insulin resistance.[11] In a previous study, we have reported that $RAG1^{-/-}$ mice fed with high-fructose diet did not develop hepatic steatosis.[12] It also showed higher insulin sensitivity and lower glucose intolerance as compared to wild-type controls. Nevertheless, not many studies have been performed on NAFLD or NASH using this strain of mice.

B Cell-deficient Models

Recent investigations have attributed important functions of B cells in various metabolic syndromes, such as insulin resistance by modulation of T cells.[13,14] B cell activating factor (BAFF) promotes B cell differentiation and expansion, consequently leading to increased immunoglobulin levels in the serum. Both $BAFF^{-/-}$ and $BAFFR^{-/-}$ mice have reduced the number of mature B cells.[15] In response to a high-fat diet, visceral adipose tissues show increased expression of BAFF, which is released into the blood stream.[16] Higher levels of serum BAFF levels are also noted in patients with hepatic steatosis.[17] A study by our group in CBA/CaHN-Btk^{xid}/J mice, having mutation in the Bruton's tyrosine kinase gene (Btk) with reduced surface IgM to IgD ratio and disorder in B-cell maturation, has shown that high-fructose diet has no influence on the progression of NAFLD when compared to wild type high-fat diet fed B6 mice.[12]

NKT Cell-deficient Mice

NK cells and NKT cells are abundant in the hepatic lymphocytes and act as a link between the innate and the adaptive immune response. NKT is a unique subset of innate immune cells expressing both T cell receptors, as well as NK cell markers, and constitute up to 30% of the hepatic lymphocytes.[18,19] The role of NK cells and NKT cells in the pathogenesis of NAFLD has been widely investigated but it remains poorly understood. In different kinds of liver diseases as hepatocarcinoma, viral hepatitis, and autoimmune liver diseases, NK cells modulate inflammation of the hepatocytes and contribute to the propagation of fibrosis.[20] There are two types of NKT cells – type I and type II.[21] Type I NKT cells, also called invariant NKT cells (iNKT cells), express an invariant TCR called Vα14 in mice and Vα24 in humans that recognize glycolipids in conjugation with CD1d. The iNKT cells are pro-inflammatory in nature, secreting large amount of TNF alpha and IFN gamma, on stimulation with a lipid antigen.[21] These pro-inflammatory cytokines in turn recruit neutrophils and macrophages in the liver, leading to NASH. Consequently, $J\alpha18^{-/-}$ mice lacking iNKT cells,[22] when fed with high-fat diet, attenuate the condition of NAFLD.[23] Also, iNKT cells play a protective role against liver inflammation progressing to fibrosis but not against steatosis, which is enhanced by dietary excess fat, suggesting a key role of these cells in NASH pathogenesis.[24] Numerous studies report an inverse correlation between NKT cell number and lipid accumulation in the hepatocytes.[20] Diet-induced hepatic steatosis also reduces the NKT cell number, thus confirming the observation.[25] On feeding with high-fat diet, CD1d knockout mice ($CD1d^{-/-}$ mice) lacking both type I and type II NKT cells,[22] displayed increased susceptibility to fat deposition in the liver, as well as hepatic inflammation.[26]

Major Histocompatibility Complex II Knockout ($MHC\ II^{-/-}$) Mice

Major histocompatibility complex II molecules, present on the surface of antigen presenting cells like macrophages,[27] are involved in presenting the processed antigen to $CD4^+$ T cells resulting in an immune response against the antigen.[28] Studies involving $MHC\ II^{-/-}$ mice, fed with high-fat diet, showed no significant difference in the intensity of hepatic steatosis, fibrosis, inflammation, and obesity as compared to wild-type controls. This clearly indicates that MHC II pathway is not involved in the development of hepatic steatosis.[29]

CD40 Knockout ($CD40^{-/-}$) Mice

CD40 is a surface molecule present on the surface of a variety of immune cells[30] and gets activated on binding to its ligand CD40L,[31] initiating a wide variety of immune responses. $CD40^{-/-}$ mice fed with high-fat diet show a decrease in the hepatic cytokine levels. However, they exhibit a higher level of liver steatosis, insulin resistance, and glucose intolerance, compared to their age-matched wild-type controls.[32]

Interferon Regulatory Factors Knockout Mouse Models

Interferon regulatory factors (IRFs) are a family of transcription factors that regulate the transcription of interferons and consist of nine members (IRF1–IRF9) in mammals.[33] Most IRFs are involved in innate immunity and defense against pathogens. The role of IRFs in the pathogenesis of NAFLD is varied, with some members of the family involved in aggravating the inflammation, while others involved in the attenuation of inflammation. Studies on $IRF7^{-/-}$ mice fed with high-fat diet indicate lower hepatic fat deposition, higher glucose tolerance, and insulin sensitivity, as well as lower weight gain as compared to wild-type controls. Thus, $IRF7^{-/-}$ condition prevents diet-induced obesity and insulin resistance.[34] In contrast, a similar study done on IRF9-deficient mice displayed higher insulin resistance, aggravated inflammation, and hepatic steatosis in response to consumption of high-fat diet.[35]

Toll-like Receptor (TLR)-deficient Mouse Models

A number of reports show the role of TLRs in the progression of NAFLD. TLRs that present on the surface of

Table 1 Mouse Models Lacking Various Immune Components and its Effect on NAFLD/NASH. Upward Facing Arrow (↑) Indicates Enhanced Condition as Compared with the Respective Wild-type Control and Downward Facing Arrow (↓) Indicates Vice Versa.

Mouse strain	Deficiency	Diet inducing NAFLD	Effects	Reference
B6.129S2-Tap1$^{tm1Arp/J}$	$CD8^+$ T cell	High-fructose diet	Hepatic steatosis ↓ Insulin sensitivity ↑ Glucose intolerance ↓ Inflammation ↓	Arindkar et al.[11], and Bhattacharjee et al.[12]
CBA/CaHN-Btk$^{xid/J}$	B cell deficient	High-fructose diet	Not involved in NAFLD	Bhattacharjee et al.[12]
C.129S2-Cd1$^{tm1Gru/J}$	CD1 deficient	High-fat diet	Hepatic steatosis ↑ Inflammation ↑	Martin-Murphy BV et al.[26]
B6.129S2-H2$^{dlAb1-Ea/J}$	MHC II deficient	High-fat diet	Not involved in NAFLD	Gilles et al.[29]
B6.129P2-Cd40$^{tm1Kik/J}$	CD40 deficient	High-fat diet	Hepatic inflammatory cytokines ↓ Liver steatosis ↑ Insulin resistance ↑ Glucose intolerance ↑	Guo et al.[32]
IRF $7^{-/-}$ mice	IRF7 deficient	High-fat diet	Hepatic inflammatory cytokines ↓ Liver steatosis ↓ Insulin sensitivity ↑ Glucose tolerance↑	Wang et al.[34]
IRF $9^{-/-}$ mice	IRF9 deficient	High-fat diet	Liver steatosis ↑ Insulin sensitivity ↓ Hepatic inflammation ↑	Wang et al.[35]
TLR $2^{-/-}$ mice	TLR2 deficient	MCD diet	Fibrosis ↑ Steatohepatitis ↑	Chantal et al.[37]
TLR $9^{-/-}$ mice	TLR9 deficient	CDAA diet	Steatohepatitis ↓ Fibrosis ↓	Brenner et al.[41]
B6.129P2(SJL) Myd88$^{tm1.1Defr/J}$	MyD88 deficient	CDAA diet	Steatohepatitis ↓ Inflammation ↓ Insulin resistance ↓	Miura et al.[42]
B6.129S2-Il6$^{tm1Kopf/J}$	IL-6 deficient	Ethanol induced	Hepatic steatosis ↑	El-Assal et al.[48]
Il17ra^{tm1Koll}	IL-17 deficient	High-fat diet	Hepatic steatosis ↓ Inflammation ↓	Tang et al. [52], and Xu et al.[55]
B6.129P2-Il10$^{tm1Cgn/J}$	IL-10 deficient	High-fat diet Ethanol induced	Hepatic steatosis ↓ Inflammation ↓	Miller A et al.[56]
IL-1Ra−/−	IL-1 deficient	High-fat diet	Hepatic steatosis ↓ Loss in weight ↑	Negrin et al.[57]
B6.129S1-Mapk8$^{tm1Flv/J}$	JNK1 deficient	CDAA diet	Steatohepatitis ↓ Fibrosis ↓	Yuzo et al.[58]
C.129S4(B6)-Mif$^{tm1Dvd/J}$	MIF deficient	High-fat diet MCD diet	Hepatic steatosis ↑ Inflammation ↑ Fibrosis ↓	Heinrichs et al.[61,62]
129-Smad3$^{tm1Par/J}$	Smad3 deficient	High-fat diet	Obesity ↓ Steatosis ↓ Glucose tolerance ↑ Insulin sensitivity ↑	Yadav et al.[63]
Cmklr1$^{-/-}$	Cmklr1 deficient	High-fat diet	Not involved in NAFLD	Gruben et al.[65]
Nlrp3$^{-/-}$	Nlrp3 deficient	High-fat diet	Prevent inflammation in liver	Vandanmagsar et al.[64]

Kupffer macrophages in the liver have been implicated in the development of NAFLD. Among various TLRs identified in mammals, 3 TLRs, viz., TLR2, TLR4, and TLR9 have been shown to be involved in NAFLD.

TLR2$^{-/-}$ Mice

TLR2 plays an important role in lipid trafficking by uptake of diacylated lipoproteins.[36,] Hence, as expected, studies on TLR2$^{-/-}$ mice fed with methionine choline-deficient (MCD) diet presented an exaggerated NASH condition with increased steatohepatitis and fibrosis.[37]

TLR4$^{-/-}$ Mice

TLR4 expressing Kupffer macrophages are activated on exposure to endotoxins, like lipopolysaccharides, and help in their elimination. Rivera et al.[38,] demonstrated the role of TLR4 in the development of steatohepatitis in methionine/choline-deficient diet. Tsukumo et al.[39,] observed activation of TLR4 and increased steatohepatitis in mice fed with high-fat diet, while Wagnerberger et al.[40] reported elevated expression levels of all the TLRs (from 1 to 9) and also the downstream adaptor Myd88, in high-fructose fed mice.

TLR9$^{-/-}$ Mice

Toll-like receptor 9 (TLR9) is a PRR that recognizes bacteria-derived cytosine phosphate guanine-containing DNA and activates innate immunity. TLR9 expressing Kupffer macrophages, on interaction with TLR9 ligands, produce IL-1B leading to lipid accumulation in the hepatocytes, subsequently causing inflammation of the hepatocytes, and cell death. Mice deficient in TLR9 have reduced steatohepatitis and fibrosis.[41]

Myd88 Knockout (Myd88$^{-/-}$) Mice

MyD88 is the downstream molecule in the TLR pathway. MyD88$^{-/-}$ mice are protected from metabolic syndrome, as well as atherosclerosis. Miura et al. demonstrated that MyD88$^{-/-}$ mice on a choline-deficient L-amino acid defined (CDAA) diet had less steatohepatitis and lower insulin resistance compared with wild-type mice.[42,] Inflammatory cytokines and fibrogenic factors are also significantly suppressed in MyD88$^{-/-}$ mice compared with wild-type mice.[42]

Cytokine-deficient Models

TNF Alpha Knockout (TNF$^{-/-}$) Mice

TNF alpha is a pro-inflammatory cytokine and plays a major role in the progression of NAFLD. Inhibition of TNF alpha has been shown to inhibit the progression of NAFLD.[43,] A number of studies correlate the serum levels of TNF alpha with the severity of NAFLD.[44] TNF alpha produced by the innate immune cells of the liver like the resident liver macrophages (Kupffer cells) has a role in the early stages of NASH progression. Kupffer macrophages are the first to reach the site of liver injury, followed by the secretion of large amounts of TNF alpha, which in turn recruit other immune cells like the CD11c^{+} dendritic cells. Targeted knockdown of TNF alpha or its receptor has been shown to reduce the severity of NASH.[45,46]

Interleukin 6 Knockout (IL-6$^{-/-}$) Mice

IL-6 is a multi-functional cytokine mainly secreted by T cells and macrophages, in response to binding of pathogen recognition receptors (PRRs) like TLRs, on the surface of immune cells to their ligands. IL-6 also promotes lipogenesis and is associated with insulin resistance,[47,] as well as impairs insulin signaling. However, the effects of IL-6 are complex and its role in liver steatosis is disputed. Studies performed on IL-6$^{-/-}$ mice by El-Assal et al.[48,] suggested the protective role of IL-6 in the prevention of hepatic steatosis by protecting suppression of ethanol-induced alcoholic stress and mitochondrial dysfunction. IL-6 was also shown to promote hepatic protection and regeneration by attenuating ischemia-reperfusion injury and promoting cell cycle of the hepatocytes.[49,50] Increase in plasma IL-6 levels have been recorded in patients with NAFLD, as well as in animal models of NAFLD.[51] Hence, IL-6$^{-/-}$ mice can be a valuable tool in understanding the role, as well as mechanism of action of IL-6 in diet-induced model of NAFLD, and potentially help in developing therapeutics.

Interleukin 17 Knockout (IL-17$^{-/-}$) Mice

IL-17 is another pro-inflammatory cytokine produced by the T helper 17 (Th17) cells. Reports suggest an increase in the proportion of hepatic Th17 cells in response to high-fat diet, thereby accelerating the inflammation of hepatocytes in NAFLD.[52,53] This can be explained by the opposing role of Th17 against Treg cells, which is responsible for the attenuation of inflammation.[54,] Numerous studies have shown an improvement in liver function, as well as a significant decrease in steatosis and inflammation of liver by suppressing IL-17 in high-fat fed mice.[55]

Interleukin 10 Knockout (IL-10$^{-/-}$) Mice

Anti-inflammatory cytokines like IL-4 and IL-10 play a hepato-protective by attenuating Th1 response. IL-10$^{-/-}$ is more susceptible to liver inflammatory response as compared to wild-type controls. However, they are resistant to hepatic steatosis and hepatocellular injury, unlike the damage usually induced by ethanol or HFD feeding to wild-type animals.[56]

Mice with Interleukin 1B Signaling Interference

IL-1B promotes deposition of fats in the liver by up-regulating the expression of fatty acid synthase. Disruption of IL-1 signaling in high-fat fed mice by treatment with IL-1 receptor antagonist (IL-1Ra) showed remarkably reduced hepatic lipid accumulation and lack of weight gain as compared to untreated animals.[57]

JNK1 Knockout (JNK$^{-/-}$) Mice

Intracellular signaling pathways are critical regulators of both the hepatic accumulation of lipid and the hepatocytic injury response in the setting of steatosis. c-Jun N-terminal kinase (JNK) is activated by oxidants, as well as cytokines, and regulates hepatocellular injury along with insulin resistance, suggesting that this kinase may play a very important role in the development of NAFLD. In mice fed with a CDAA diet, JNK1$^{-/-}$ and JNK2$^{-/-}$ mice showed significantly reduced levels of fibrosis and inflammation in the liver as compared to wild-type controls.[58,] JNK1 is responsible for JNK activation that promotes the development of steatohepatitis in the MCD diet model.[59]

Macrophage Inhibitory Factor Knockout (MIF$^{-/-}$) Mice

The macrophage migration inhibitory factor is a pro-inflammatory cytokine that is involved in recruiting macrophages to the site of injury and consequently leading to inflammation. It acts via the invariant chain (CD74)—AMPK pathway[60,]—and connects the adaptive immune response. MIF$^{-/-}$ mice fed with either MCD diet or a high-fat diet showed elevated expression of lipogenic genes, consequently leading to lipid accumulation in the liver, as well as increased hepatic inflammation, suggesting a hepato-protective role of MIF.[61,] Numerous reports also supported an antifibrotic role of MIF.[62]

Smad3$^{-/-}$ Mice

Smad3 is a key mediator in TGF-β signaling and is involved in regulating glucose and energy homeostasis. On high-fat fed diet, smad3-deficient mice showed less weight gain, enhanced glucose tolerance, and insulin sensitivity and were protected from hepatic steatosis as compared to wild-type controls. Smad3$^{-/-}$ mice are also protected from diet-induced obesity, diabetes, and steatosis.[63]

Nlrp3 Knockout (Nlrp3$^{-/-}$) Mice

Nod-like receptors (NLRs) can cooperate with TLRs and regulate inflammatory and apoptotic response. They are found in lymphocytes, macrophages, dendritic cells, and also in non-immune cells, for example in the epithelium. Nlrp3 inflammasome regulates insulin-sensitivity and steatohepatitis in obesity and prevents the development of steatohepatitis on mice fed with high-fat diets.[64]

Cmklr1 Knockout (Cmklr1$^{-/-}$) Mice

High-fat fed mice deficient in Chemokine-like receptor 1 (Cmklr1), which is a receptor for the adipokine, showed no difference in the expression of hepatic inflammatory genes, as well as lipid accumulation and immune cell infiltration of the liver, as compared to the wild-type controls.[65]

Cross Talk Between Immune Cells in the Development of NAFLD/NASH

It is increasingly becoming clear that there are complex immune and inflammatory pathways that interact and participate in the progression of NASH, involving signaling in various cell types that are stimulated by PAMPs and DAMPs, as well as interaction between different cell types and tissues. After binding of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) to PRRs, the liver innate immune cells [Kupffer cells, monocytes, neutrophils, dendritic cells (DCs), NK cells, and NK T (NKT) cells] initiate and maintain hepatic inflammation through cytokine production.[66,67] Using various mouse models (Table 1), existence of cross talk between immune cells, the resident parenchymal cell, and various signaling pathways during the innate immune response, as well as cellular responses upon liver inflammation, has been determined. Nevertheless, many studies are required, particularly with immune-deficient mouse models, for understating the pathophysiology of NAFLD/NASH and for developing appropriate therapeutics.

CONCLUSION

The pathogenesis of NAFLD is a very complex process involving various immune cells, as well as non-immune cells. Moreover, the underlying signaling pathways activated in parenchymal and non-parenchymal liver cells promoting NASH and facilitating the transition from NASH to HCC remain poorly understood. The ultimate onset of NAFLD is perceived to be caused by a disturbance in the intricate balance of various cytokines, as well as signaling pathways maintained by the immune cells. This review provides an elaborate insight of the role played by various immune components by the use of knockout/knockdown animal models lacking that particular component. Knowledge from such important animal models can help in understanding various pathways that can be targeted for developing drugs for the treating NAFLD.

CONFLICTS OF INTEREST

The authors have none to declare.

REFERENCES

1. Bugianesi E, McCullough AJ, Marchesini G. Insulin resistance: a metabolic pathway to chronic liver disease. *Hepatology.* 2005;42:987–1000.
2. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc.* 1980;55:434–438.
3. Judith E, Alexander D, Jan-Peter S, et al. Non-alcoholic fatty liver disease progresses to hepatocellular carcinoma in the absence of apparent cirrhosis. *Epidemiology.* 2011;128:2436–2443.

4. Bellentani S, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. *Dig Dis.* 2010;28(1):155–161.
5. Paschos P, Paletas K. Non alcoholic fatty liver disease and metabolic syndrome. *Hippokratia.* 2009;13(1):9–19.
6. Keith G, Anthony S. Treatment of non-alcoholic fatty liver disease. *Ther Clin Risk Manag.* 2007;3(December (6)):1153–1163.
7. Bugianesi E, Leone N, Vanni E, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology.* 2002;123:134–140.
8. Marchesini G, Bugianesi E, Forlani G, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology.* 2003;37:917–923.
9. Wolf MJ, Adili A, Piotrowitz K, et al. Metabolic activation of intrahepatic CD8+ T cells and NKT cells causes nonalcoholic steatohepatitis and liver cancer via cross-talk with hepatocytes. *Cancer Cell.* 2014;26:549–564.
10. Nishimura S, Manabe I, Nagasaki M, et al. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat Med.* 2009;15:914–920.
11. Arindkar S, Bhattacharjee J, Kumar JM, et al. Antigen peptide transporter 1 is involved in the development of fructose-induced hepatic steatosis in mice. *J Gastroenterol Hepatol.* 2013;28: 1403–1409.
12. Bhattacharjee J, Kumar JM, Arindkar S, et al. Role of immunodeficient animal models in the development of fructose induced NAFLD. *J Nutr Biochem.* 2014;25:219–226.
13. Duffaut C, Galitzky J, Lafontan M, Bouloumie A. Unexpected trafficking of immune cells within the adipose tissue during the onset of obesity. *Biochem Biophys Res Commun.* 2009;384:482–485.
14. Winer DA, Winer S, Shen L, et al. B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. *Nat Med.* 2011;17:610–617.
15. Sasaki Y, Casola S, Kutok JL, Rajewsky K, Schmidt-Supprian M. TNF family member B cell-activating factor (BAFF) receptor-dependent and -independent roles for BAFF in B cell physiology. *J Immunol.* 2004;173:2245–2252.
16. Hamada M, Abe M, Miyake T, et al. B cell-activating factor controls the production of adipokines and induces insulin resistance. *Obesity (Silver Spring).* 2011;19:1915–1922.
17. Teruki M, Masanori A, Yoshio T, et al. B-cell activating factor is associated with the histological severity of nonalcoholic fatty liver disease. *Hepatol Int.* 2013;7(June (2)):539–547.
18. Gao B, Radaeva S, Park O. Liver natural killer and natural killer T cells: immunobiology and emerging roles in liver diseases. *J Leukoc Biol.* 2009;86:513–528.
19. Exley MA, Koziel MJ. To be or not to be NKT: natural killer T cells in the liver. *Hepatology.* 2004;40:1033–1040.
20. Zhan YT, An W. Roles of liver innate immune cells in nonalcoholic fatty liver disease. *World J Gastroenterol.* 2010;16:4652–4660.
21. Tajiri K, Shimizu Y. Role of NKT cells in the pathogenesis of NAFLD. *Int J Hepatol.* 2012;2012:850836.
22. Martin-Murphy BV, You Q, Wang H, et al. Mice lacking natural killer T cells are more susceptible to metabolic alterations following high fat diet feeding. *PLoS ONE.* 2014;9(1):e80949.
23. Deng ZB, Liu Y, Liu C, et al. Immature myeloid cells induced by a high-fat diet contribute to liver inflammation. *Hepatology.* 2009;50: 1412–1420.
24. Takuya M, Tetsuo T, Akio U, et al. Absence of invariant natural killer T cells deteriorates liver inflammation and fibrosis in mice fed high-fat diet. *J Gastroenterol.* (45):2010;(45):1247–1254.
25. Li Z, Soloski MJ, Diehl AM. Dietary factors alter hepatic innate immune system in mice with nonalcoholic fatty liver disease. *Hepatology.* 2005;42:880–885.
26. Martin-Murphy BV, You Q, Wang H, et al. Mice lacking natural killer T cells are more susceptible to metabolic alterations following high fat diet feeding. *PLoS One.* 2014;9:e80949.
27. Blum JS, Wearsch PA, Cresswell P. Pathways of antigen processing. *Annu Rev Immunol.* 2013;31:443–473.
28. Mitchison NA. T-cell-B-cell cooperation. *Nat Rev Immunol.* 2004;4:308–312.
29. Gilles W, Catherine R, Armelle B, Kaori M. Major histocompatibility class II pathway is not required for the development of nonalcoholic fatty liver disease in mice. *Int J Endocrinol.* 2013;2013:.
30. Grewal IS, Flavell RA. CD40 and CD154 in cell-mediated immunity. *Annu Rev Immunol.* 1998;16:111–135.
31. Banchereau J, Bazan F, Blanchard D, et al. The CD40 antigen and its ligand. *Annu Rev Immunol.* 1994;12:881–922.
32. Guo CA, Kogan S, Amano SU, et al. CD40 deficiency in mice exacerbates obesity-induced adipose tissue inflammation, hepatic steatosis, and insulin resistance. *Am J Physiol Endocrinol Metab.* 2013;304:E951–E963.
33. Savitsky D, Tamura T, Yanai H, Taniguchi T. Regulation of immunity and oncogenesis by the IRF transcription factor family. *Cancer Immunol Immunother.* 2010;59:489–510.
34. Wang XA, Zhang R, Zhang S, et al. Interferon regulatory factor 7 deficiency prevents diet-induced obesity and insulin resistance. *Am J Physiol Endocrinol Metab.* 2013;305:E485–E495.
35. Wang X-A, Zhang R, Dingsheng J, et al. Interferon regulatory factor 9 protects against hepatic insulin resistance and steatosis in male mice. *Hepatology.* 2013;58(August (2)):603–616.
36. Hoebe K, Georgel P, Rutschmann S, et al. CD36 is a sensor of diacylglycerides. *Nature.* 2005;433:523–527.
37. Chantal A, LaTausha G, Monique A, et al. Toll-like receptor-2 deficiency enhances non-alcoholic steatohepatitis. *BMC Gastroenterol.* 2010;10:52.
38. Rivera CA, Adegboyega P, van RN, Tagalicud A, Allman M, Wallace M. Toll-like receptor-4 signaling and Kupffer cells play pivotal roles in the pathogenesis of non-alcoholic steatohepatitis. *J Hepatol.* 2007;47:571–579.
39. Tsukumo DM, Carvalho-Filho MA, Carvalheira JB, et al. Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance. *Diabetes.* 2007;56:1986–1998.
40. Wagnerberger S, Spruss A, Kanuri G, et al. Toll-like receptors 1–9 are elevated in livers with fructose-induced hepatic steatosis. *Br J Nutr.* 2012;107:1727–1738.
41. Brenner DA, Seki E, Taura K, et al. Non-alcoholic steatohepatitis-induced fibrosis: Toll-like receptors, reactive oxygen species and Jun N-terminal kinase. *Hepatol Res.* 2011;41:683–686.
42. Miura K, Kodama Y, Inokuchi S, et al. Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1beta in mice. *Gastroenterology.* 2010;139:323–334.
43. Chu CJ, Lu RH, Wang SS, et al. Risk factors associated with non-alcoholic fatty liver disease in Chinese patients and the role of tumor necrosis factor-alpha. *Hepatogastroenterology.* 2007;54: 2099–2102.
44. Hui JM, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? *Hepatology.* 2004;40:46–54.
45. Feldstein AE, Werneburg NW, Canbay A, et al. Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression via a lysosomal pathway. *Hepatology.* 2004;40(1):185–194.
46. Tosello-Trampont AC, Landes SG, Nguyen V, Novobrantseva TI, Hahn YS. Kupffer cells trigger nonalcoholic steatohepatitis development in diet-induced mouse model through tumor necrosis factor-α production. *J Biol Chem.* 2012;287(48):40161–40172.
47. Dogru T, Ercin CN, Erdem G, Sonmez A, Tapan S, Tasci I. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol.* 2008;103:3217–3218.
48. El-Assal O, Hong F, Kim WH, Radaeva S, Gao B. IL-6-deficient mice are susceptible to ethanol-induced hepatic steatosis: IL-6 protects against ethanol-induced oxidative stress and mitochondrial permeability transition in the liver. *Cell Mol Immunol.* 2004;1:205–211.

49. Hong F, Radaeva S, Pan HN, Tian Z, Veech R, Gao B. Interleukin 6 alleviates hepatic steatosis and ischemia/reperfusion injury in mice with fatty liver disease. *Hepatology.* 2004;40:933–941.
50. Teoh N, Field J, Farrell G. Interleukin-6 is a key mediator of the hepatoprotective and pro-proliferative effects of ischaemic preconditioning in mice. *J Hepatol.* 2006;45:20–27.
51. Wieckowska A, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol.* 2008;103(6): 1372–1379.
52. Tang Y, Bian Z, Zhao L, et al. Interleukin-17 exacerbates hepatic steatosis and inflammation in non-alcoholic fatty liver disease. *Clin Exp Immunol.* 2011;166:281–290.
53. Harley IT, Stankiewicz TE, Giles DA, et al. IL-17 signaling accelerates the progression of nonalcoholic fatty liver disease in mice. *Hepatology.* 2014;59:1830–1839.
54. Eisenstein EM, Williams CB. The T(reg)/Th17 cell balance: a new paradigm for autoimmunity. *Pediatr Res.* 2009;65:26R–31R.
55. Xu R, Tao A, Zhang S, Zhang M. Neutralization of interleukin-17 attenuates high fat diet-induced non-alcoholic fatty liver disease in mice. *Acta Biochim Biophys Sin (Shanghai).* 2013;45:726–733.
56. Miller AM, Wang H, Bertola A, et al. Inflammation-associated interleukin-6/signal transducer and activator of transcription 3 activation ameliorates alcoholic and nonalcoholic fatty liver diseases in interleukin-10-deficient mice. *Hepatology.* 2011;54:846–856.
57. Negrin KA, Roth Flach RJ, DiStefano MT, et al. IL-1 signaling in obesity-induced hepatic lipogenesis and steatosis. *PLoS One.* 2014;9:e107265.
58. Yuzo K, Tatiana K, Keiko I, et al. JNK1 from hematopoietic cells mediates progression from hepatic steatosis to steatohepatitis and fibrosis in mice. *Gastroenterology.* 2009;137(October (4)): 1467–1477.e5.
59. Schattenberg JM, Singh R, Wang Y, et al. JNK1 but not JNK2 promotes the development of steatohepatitis in mice. *Hepatology.* 2006;43:163–172.
60. Miller EJ, Li J, Leng L, et al. Macrophage migration inhibitory factor stimulates AMP-activated protein kinase in the ischaemic heart. *Nature.* 2008;451:578–582.
61. Heinrichs D, Berres ML, Coeuru M, et al. Protective role of macrophage migration inhibitory factor in nonalcoholic steatohepatitis. *FASEB J.* 2014;28:5136–5147.
62. Heinrichs D, Knauel M, Offermanns C, et al. Macrophage migration inhibitory factor (MIF) exerts antifibrotic effects in experimental liver fibrosis via CD74. *Proc Natl Acad Sci USA.* 2011;108: 17444–17449.
63. Yadav H, Quijano C, Kamaraju AK, et al. Protection from obesity and diabetes by blockade of TGF-beta/Smad3 signaling. *Cell Metab.* 2011;14:67–79.
64. Vandanmagsar B, Youm YH, Ravussin A, et al. The NLRP3 inflammasome instigates obesity-induced inflammation and insulin resistance. *Nat Med.* 2011;17:179–188.
65. Gruben N, Aparicio VM, Kloosterhuis NJ, et al. Chemokine-like receptor 1 deficiency does not affect the development of insulin resistance and nonalcoholic fatty liver disease in mice. *PLoS One.* 2014;9:e96345.
66. Bieghs V, Trautwein C. The innate immune response during liver inflammation and metabolic disease. *Trends Immunol.* 2013;34: 446–452.
67. Michal G, Gyongyi S. Immune and inflammatory pathways in NASH. *Hepatol Int.* 2013;7(December (suppl 2)):771–781.