A high-trans fat, high-carbohydrate, high-cholesterol diet-induced nonalcoholic steatohepatitis mouse model and its hepatic immune response

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Research Article

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a chronic progressive disease that can progress to non-alcoholic steatohepatitis (NASH). Animal models are important tools for basic NASH research. Immune activation plays a key role in liver inflammation in patients with NASH. We established a high-trans fat, high-carbohydrate, and high-cholesterol diet-induced (HFHCC) mouse model. C57BL/6 mice were fed a normal or HFHCC diet for 24 weeks, and the immune response characteristics of this model were evaluated. The results showed that mice treated with HFHCC diet exhibited remarkably increased hepatic triglycerides (TG) content, and the increase in plasma transaminases resulted in hepatocyte injury. Biochemical results showed that HFHCC induced glucose and lipid metabolism disorders; marked hepatocyte steatosis, ballooning, inflammation, and fibrosis. The proportion of innate immunity-related cells, including Kupffer cells (KCs), neutrophils, dendritic cells (DCs), natural killer T cells (NKT), and adaptive immunity-related CD3 + T cells increased; interleukin-1α (IL-1α), IL-1β, IL-2, IL-6, IL-9, and chemokines, including CCL2, CCL3, and macrophage colony stimulating factor (G-CSF) increased. The constructed model closely approximated the characteristics of human NASH and evaluation of its immune response signature, showed that the innate immune response was more pronounced than adaptive immunity. Its use as an experimental tool for understanding innate immune responses in NASH is recommended.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is gradually becoming one of the most frequent liver diseases worldwide. NAFLD includes a broad spectrum of diseases ranging from nonalcoholic fatty liver to nonalcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma[1]. In recent years, the incidence of NASH-related mortality has rapidly increased. Research on the pathogenesis of NASH and drug development has been conducted like a raging fire. For this process, a suitable animal model is required. Animal models that are as similar as possible to human NASH disease characteristics are needed for pathogenesis and drug development.

Excessive energy intake and insufficient energy expenditure are the main factors leading to the occurrence of fatty liver. An animal model based on diet-induced excess nutrition is one of the most common NAFLD models. A high-fat diet is currently the most classic and commonly used NAFLD model. Commonly used fats mainly include saturated fats, such as lard and tallow, and unsaturated fats, such as soybean oil. In addition to high fat, high sugar is the main factor that induces the occurrence and development of fatty liver. Carbohydrates, such as fructose and sucrose, are not only stimulate de novo lipogenesis but also induce hepatic oxidative stress and inflammation[2]. Compared with the simple high-fat diet, high fat combined with high sugar intake requires a relatively short time for modeling, and the degree of disease is relatively severe[3]. Although conventional high-fat and high-sugar diets can induce pathological features of NASH, the fibrotic features of the model are not obvious. Researchers have attempted to add other ingredients to accelerate disease and fibrosis progression.
Trans fat is an unsaturated fat that can lead to increased expression of lipogenic genes in the liver and enhanced phagocytosis of KCs[4], which can exacerbate steatosis, inflammation, and fibrosis[5]. Cholesterol plays an important role in the progression of NAFLD to NASH, which has been shown to exacerbate progression[6,7]. Adding bile salts to high-cholesterol feeds can enhance cholesterol absorption and inhibit cholesterol clearance, which is conducive to the deposition of fat in the body. Many studies have confirmed the role of high trans fatty acids, high sugars, and high cholesterol in NASH models, but few studies have involved the simultaneous use of high-trans fat, high-carbohydrate, high-cholesterol (HFHCC) models to induce NASH. Therefore, in this study, we aimed to explore an animal model that is close to the histological characteristics of patients with NASH and simultaneously shows more prominent NASH characteristics. High fat diets containing trans fats with increased cholesterol and bile salts were used in combination with high-fructose drinking water for 25 weeks to induce a mouse model of NASH.

Evidence is mounting that the immune response plays an important role in the progression of NASH[8]. Glycolipid metabolism disorders caused by high fat and sugar levels can induce liver immune disorders and inflammation. Immune disorders aggravate glucose and lipid metabolism disorders, forming a vicious circle[9]. Immune responses include innate and adaptive immunity, both of which are involved in NASH-related inflammation. For the purpose of revealing the formation mechanism of the model better, especially the immune mechanism, we conducted a comprehensive and systematic analysis of the immunological characteristics of the model as a whole to provide a basis for clarifying the immune mechanism of the model.

Materials And Methods

Five-week-old male C57BL/6 mice, purchased from Shanghai Slack Laboratory Animal Center (Shanghai, China), were group-housed in the Animal Center of Shanghai University of Traditional Chinese Medicine. Mice were maintained in a 12:12-h light-dark cycle schedule. Mice were randomly divided into either normal diet (10% fat, Trophic Animal Feed High-tech Co., Ltd, China, TP23301S) and normal water or high-fat, high-carbohydrate, and high bile salt diet (HFHCC) (45% of the feed energy was derived from fat, trophic Animal Feed High-tech Co., Ltd, China, TP23302S), and drinking water enriched with high fructose. Sugar water with a concentration of 42 g/L was made from drinking water at a ratio of 55% fructose and 45% sucrose by weight. The composition of each diet is presented in Supplementary Tables 1 and 2. The animals were provided ad libitum access to the diet for 25 weeks. All animals were sacrificed for tissue collection at the end of the 25th week. All animal procedures were performed in line with the National Institutes of Health Guidelines for Laboratory Animals and were approved by the Animal Ethics Committee of Shanghai University of Chinese Medicine.

Biochemical Assays
Animals were sacrificed after 25 weeks, and serum was obtained by taking the eyeballs after an overnight fast. Serum glucose (GLU) was measured with a glucose quantification kits according to the manufacturer's instructions (Nanjing Jian Cheng Bioengineering Institute, Nanjing, China). Serum insulin levels were determined using an ultra-sensitive mouse insulin ELISA kit (18APUMI482A). The hepatic liver triglyceride (TG) content was obtained from triglyceride kit according to the manufacturer's instructions (Dongou Bioengineering, Zhejiang, China) at 25 weeks. Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels were detected using a TOSHIBA TBA-40FR Automatic Analyzer (Hitachi, Limited, Tokyo, Japan).

**Histology**

Liver tissues were collected from the mice after 25 weeks, fixed in 10% formalin, paraffin embedded, and sectioned into 4 µm slices. Tissue sections were differentiated with 1% hydrochloric acid alcohol for 5–10 s and then stained with hematoxylin and eosin (H&E). Sirius Red was used to visualize the degree of fibrosis and collagen deposition. Analysis was performed using Sirius red-stained area aperture image scope-pathology slide viewing software. Liver fibrosis stage was determined using a previously published fibrosis staging system[10].

**Immunohistochemistry**

Liver tissues were collected from the mice after 25 weeks and fixed in 10% formalin. Immunostaining was performed using 7 µm formalin-fixed, paraffin-embedded sections. Deparaffinized tissue sections were subjected to antigen retrieval. Col-1 (collagen type-1) and sodium citrate buffer were used for antigen retrieval of α-SMA (α-smooth muscle actin) in liver tissue sections. The repair method for MPO was the high-pressure repair method using sodium citrate buffer. The method used for F4-80 was the EDTA high-pressure repair method. Endogenous peroxidase activity was quenched. Mouse monoclonal anti-PAR antibody (Trevigen, Gaithersburg, MD) was used at a 1:300 dilution overnight at 4°C, and then mixed with goat anti-mouse F4/80 (KCs marker) (1:100, Abcam, ab111101), MPO (myeloperoxidase, neutrophil marker) (1:100, Abcam, 22225-1-AP), COL-1 (1:100, Abcam, ab34710), α-SMA (1:100, Abcam, ab5694), and biotinylated goat anti-rabbit IgG (Biotech Well, WH1057-2), followed by signal amplification with streptavidin and final counterstaining with hematoxylin.

**Flow Cytometry**

Single liver cell suspensions were centrifuged at 1500 rpm, the suspension was divided into three layers, and the milky white membrane layer in the middle was the lymphocytes, then the lymphocytes were separated and resuspended in 70% Percoll. Cell suspension was centrifuged at 2000 rpm for 5 min at room temperature, and then 1*10^6 cells from each sample were drawn and suspended in 29.5 µl 0.2% BSA-PBS buffer, blocked with anti-mouse CD16/32 (Becton, Dickinson and company, 553141), and incubated at 4°C for 15 min. Cells were stained with FITC-CD3 (Becton, Dickinson and Company, 553061),
PerCP-CD4 (Becton, Dickinson and Company, 553052), PE-Cy7-CD8 (Becton, Dickinson and company, 552877), APC-Cy7-CD19 (Becton, Dickinson and Company, 557655), APC-NK1.1 (Becton, Dickinson and Company, 550627), and PE-CF594-CD11C (5552454; Becton, Dickinson and Company, USA). Cells were acquired on a Beckman Flow Cytometer (BECKMAN, COULTER, DEFLEX), and the data were analyzed using FlowJo software version 7.6 (TreeStar, Ashland, OR).

Detection Of Cytokine Protein Levels In Liver Tissue

Multiplex bead immunoassay and Luminex technology (Bio-PlexPro Mouse Cytokine7-plex panel, Bio-Rad) were used to determine the cytokine levels in the mouse liver. The following 23 cytokines: interleukin-like pro-inflammatory cytokines including interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-9 (IL-9), interleukin-12 (P40) (IL-12 (P40)), interleukin-12 (P70) (IL-12 (P70)), interleukin-17A (IL-17A), interleukin-like anti-inflammatory cytokines including interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-10 (IL10), interleukin-13 (IL13); tumor necrosis factor (TNF-α), γ-Interferon (IFN-γ); chemokine including c-c motif chemokine 2 (CCL2), c-c motif chemokine 3 (CCL3), c-c motif chemokine 4 (CCL4), c-c motif chemokine 5 (CCL5), c-c motif chemokine 11 (CCL11), C-X-C motif chemokine 2 (CXCL2); colony stimulating factor including granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (G-CSF) and pluripotent colony stimulating factor (IL-3) could simultaneously be analyzed by an immunoassay kit. After liver tissue trituration and homogenization, all samples were diluted 1:2 with lysis buffer (Bio-Rad Laboratories, Hercules, CA, USA). After sonication, Supernatant Protein samples were diluted 1:3 with Bio-Plex Sample Diluent and used for multiplexing at protein concentrations of approximately 3–4 mg/mL. Finally, the beads were loaded onto a Bio-Plex 200 system (Bio-Rad) for analysis.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 8.0 GraphPad Software, La Jolla, CA). Data are expressed as mean ± standard error of measurement (SEM). Data were analyzed using test-test for comparisons of two groups. For comparison of more than two groups, ANOVA multiple comparison test was used, and p < 0.05 was regarded statistically significant.

Results

Total calories, body weight, liver weight, liver body ratio, and glucose and lipid metabolism disorders

After 25 weeks of HFHCC administration, the total calories consumed did not differ between the control and HFHCC groups (Table 1), and the body weight in the HFHCC group, was lower than that in the control group ( P 0.01, Table 1). By contrast, HFHCC exposure increased liver weight and liver body ratio (P < 0.01, Table 1). Notably, CIH exposure leads to abnormal glucose metabolism in the liver. The fasting blood-glucose (FBG) was higher in HFHCC group than in control group (P < 0.01, Table 1). The fasting insulin
level was also higher in HFHCC group compared to control group (P < 0.05, Table 1). In terms of lipid metabolism, there were no significant differences in the serum TG and TC levels between the two groups (Table 1). LDL-C in liver tissue was significantly increased in the HFHCC group than in control group (P 0.05, Table 1) and there was a trend towards decreased HDL-C in the HFHCC group (Table 1).

**Hfhcc Induced Hepatic Steatosis, Inflammation And Fibrosis**

Hematoxylin and eosin (H&E) staining showed substantial liver steatosis with inflammatory changes in the HFHCC group, and micro-and macrovesicular steatosis were clearly visible after 25 weeks. Almost all hepatocytes were enlarged owing to excessive lipid droplet deposition, and inflammatory cell infiltration was observed in the liver tissues. Sirius red staining showed remarkable liver fibrosis in the HFHCC group (Fig. 1a), and fibrosis was widespread in most portal areas. Immunohistochemical (IHC) staining of liver tissue showed that the protein expression of COL-1 and α-SMA was remarkably increased in the HFHCC group (Fig. 1a). Moreover, the collagen-positive staining area in the HFHCC group was significantly increased (P 0.01, Fig. 1c), and the fibrosis score was approximately 1.5 points (P 0.01 vs control, Fig. 1d). The steatosis, ballooning, inflammation, and NAFLD activity (NAS) scores in the HFHCC group were significantly higher than those in the control group (P 0.01, Fig. 1e). Liver tissue TG levels were higher in the HFHCC group than in the control group (Fig. 1b). In addition to histological assessment, we also examined markers of liver damage associated with NASH. Plasma ALT and AST levels also showed significant increase in HFHCC treat mice. (P < 0.01, Fig. 1f, g)

**Hfhcc Induced Changes In Innate Immunity**

Liver tissue IHC staining showed more positive F4/80 staining in the perivenous hepatic sinusoids in the HFHCC group than in the control group (Fig. 2a), and there was a large number of neutrophils around the inflammatory foci (Fig. 2a). There was no significant difference in the proportion of natural killer (NK cells) in the liver tissue between the two groups by flow cytometry assays, whereas the proportion of DCs and NKT cells were striking increased in the HFHCC group compared to those in control group. (P < 0.05, Fig. 2b).

**Hfhcc Induced Changes In Adaptive Immunity**

The liver immune cell flow cytometry assay showed that CD3 T cells were upregulated, while no significant changes in the proportion of B cells, CD4+ T cells, and CD8+ T cells between the two groups were observed (P 0.01, Fig. 3a).

**Hfhcc Induced Changes In Hepatic Cytokine Levels**
Figure 4a showed a heatmap of the 23 cytokines in the liver tissue. Compared to control group, the cytokines in the HFHCC treatment group showed an overall increasing trend. We further analyzed the changes in the levels of these 23 cytokines. The protein expression of interleukin-like pro-inflammatory cytokines IL-1α, IL-1β, IL-6, and IL-9 was strikingly upregulated in the HFHCC group (P < 0.01, vs. control, Fig. 4b), and the protein expression of IL-2 was observably upregulated in the HFHCC group (P < 0.05, vs. control, Fig. 4b), whereas the expression of interleukin-like anti-inflammatory cytokine IL-5 was downregulated in the HFHCC group (P < 0.05, Fig. 4b). There were no significant differences in IL-12 (P40), IL-12 (P70), IL-17A, and IL-13 levels between the two groups (Fig. 4b). HFHCC-diet treated mice exhibited elevated TNF-α and IFN-γ levels relative to mice on control diet. (P < 0.05; Fig. 4c). The protein expression of chemokines CCL2 and CCL3 was significantly increased in the HFHCC group (P < 0.01 vs control, Fig. 4d), whereas no notable changes in CCL4, CXCL5, CXCL2, and CCL11 were found between the two groups (Fig. 4d). Regarding colony-stimulating factors, the HFHCC group exhibited significantly increased expression of hepatic G-CSF (P < 0.05, vs. control, Fig. 4e). No significant differences in hepatic GM-CSF and IL-3 levels were observed between the two groups (Fig. 4e).

**Discussion**

Our study showed that NASH mice induced by a high-fat, high-sugar diet with trans-fat as the main fat for 25 weeks with an increased ratio of cholesterol and bile salts exhibited advanced liver inflammation, glucose and lipid metabolism disorders, and fibrosis. After the HFHCC diet model, significant changes were observed in the levels of innate immune cells and their released cytokines in the liver of mice, while the related changes in adaptive immune cells were not obvious. This model is more inclined to trigger an innate immune response than an adaptive immune response.

The liver TG content was 2.5-folds higher in the HFHCC mice at 25 weeks, and serum ALT and AST activities were significantly increased. Liver pathological analysis showed that the HFHCC diet induced hepatocytes in mice with different degrees of balloon-like changes after 25 weeks. The steatotic hepatocytes were swollen in round or oval shapes, and there are large amounts of fat vacuils within the hepatocytes. Macrophages could be observed in the hepatic sinuses, and neutrophil and monocyte infiltrates could be seen within the lobules of the liver. The mean NAS score was 4.25 points. In terms of fibrosis, col-1 positivity and α-sma positivity were significantly increased in mouse liver tissue, suggesting significant stellate cell activation and hepatic collagen deposition. Hepatic fibrosis was staged as F1-2. In terms of glucose metabolism, compared with the normal group, the glucose, insulin, and insulin resistance indices of the HFHCC model mice were significantly increased, showing obvious glucose metabolism disorder, which is very similar to the characteristics of glucose metabolism disorder in human patients with NASH.

C57BL/6J mice fed a conventional high-fat diet (60% energy from fat) for 10 weeks exhibited obesity and disturbances in glucose and lipid metabolism, developed steatosis and inflammation at 16 weeks, and showed mild fibrosis at 50 weeks[11]. C57BL/6 mice fed a high-fat, high-sugar diet for 30 weeks showed NASH characteristics, such as ballooning, glucose and lipid metabolism disorders, liver damage, and
inflammation, but the degree of fibrosis was very mild[7]. Compared with conventional high-fat high-sugar diet models, the HFHCC diet induces more pronounced and quicker pathological features of NASH. Tu et al[12]. also used a high-fat and high-cholesterol bile salt diet to induce NASH, in which 37.1% of the energy came from fat, the cholesterol content was 1.25%, and the sodium cholate content was 0.5%. The pathological results showed that the diet was modeled, and post-mice showed steatosis and inflammation but very low levels of fibrosis. On the basis of Tu’s study, our study increased cholesterol content (2%) and added high-sugar drinking water to further accelerate NASH progression, inducing more pronounced glucose and lipid metabolism disorders, and the appearance of fiber change. Similar to our study, Mells et al. found that C57BL/6J mice fed a high-trans-fat, high-sucrose, and high-cholesterol diet not only developed steatosis, lobular inflammation, and hepatocyte ballooning but also exhibited fibrosis. Together, our experiments demonstrated that the HFHCC diet could replicate the NASH model with similar metabolic and histological features in humans[13].

However, it was worth noting that although the HFHCC diet-treated mice showed significantly increased liver weight and liver body ratio compared to control diet-treated mice, yet mice in the HFHCC group exhibited significant decreased body weight by approximately 9% at 25 weeks compared to that in the Control group. A possible reason is that bile salts can participate in the metabolism of bile acids and promote the metabolism and absorption of cholesterol, activate G protein-coupled receptor 5, induce the activity of type 2 deiodinase, promote the conversion of tetraiodothyronine to triiodothyronine, and finally promote the energy expenditure, resulting in weight loss[14]. In conclusion, this model could simulate the entire disease course of human NASH patients, while exhibiting the characteristics of NASH fibrosis, showing characteristics of patients with NASH, especially non-obese patients with NASH liver fibrosis.

To further analyze the immune disorder state of this model, flow cytometry and immunohistochemistry were used to measure the proportion of immune cells in the liver, and multiplex bead immunoassay and luminex technology was used to detect the protein levels of related cytokines in liver tissue. Our findings supported that the HFHCC diet activated the liver immune response, compared with the control group, the proportions of innate immune cells such as KCs, neutrophils, and DCs were evidently increased in the model group, while the proportion of adaptive immunity-related cells, only CD3+ T cells, was higher in the model group. There were no differences in the proportions of B cells, CD4+ T cells, and CD8+ T cells between the two groups. The protein levels of pro-inflammatory cytokines, chemokines, and colony-stimulating factors released by innate immune-related cells in the liver of HFHCC mice were significantly increased, whereas the changes in inflammatory factors released by adaptive immune cells were not obvious. These results suggest that the HFHCC diet is more likely to activate innate immunity in mice.

There is mounting evidence that innate immunity plays an important role in the pathogenesis of NASH. Liver innate immune cells include KCs, neutrophils, DC, and NK[8]. KC cells are the first line of defense in the liver. When the HFHCC diet induces disorders of glucose and lipid metabolism, lipotoxicity caused by excessive accumulation of lipids in hepatocytes activates macrophages, leading to TNF-α, IL-6, and IL-1β, which further promotes liver steatosis, inflammation, and liver fibrosis[15]. In this study, immunohistochemical results showed that the HFHFF diet induced the activation of KCs. At the same
time, the pro-inflammatory cytokines (TNF-α, IL-1α, IL-1β, and IL-6) and chemokines (including CXCL1, CCL2, CCL11), and the levels of colony-stimulating factor recruitment (GM-CSF, G-CSF) released by KCs were also significantly increased. Neutrophils are another major effector of the innate immunity. The accumulation of metabolites induces the recruitment of neutrophils in the peripheral blood to the liver. Neutrophils activate macrophages, which increases the secretion of adhesion molecules and recruits neutrophils. More white blood cells enter the liver, triggering an inflammatory response. This study showed that neutrophils were significantly recruited in the liver tissue of mice in the model group, and at the same time, the levels of inflammatory factors IL1-α and G-CSF released by neutrophils were significantly increased. The role of DCs as antigen-presenting cells in NASH is complex and controversial, as hepatic DCs play a pro-inflammatory role in thioacetamide diet-induced liver fibrosis[16,17]. In the MCD diet, the reduction in hepatic DCs induces the production of inflammatory factors such as IL-6 and TNF-α, and the decrease in hepatic DCs is a type of damage to the liver[18,19]. Our study found that the number of DCs in the model group was higher than that in the normal group, and the levels of IL-10 and IL-12, two cytokines released by DCs, were significantly increased. The differences in the experimental results of the different studies may be related to the NASH modeling method. NKT cells express the surface markers CD3 of T cells and the surface markers of NK cells. These are the bridges between innate and adaptive immunity. Once recognized by antigens, they can rapidly secrete pro-inflammatory or anti-inflammatory inflammatory factors such as IFNγ and IL-4. Michael et al[20–22]. found increased numbers of NKT cells in the liver and peripheral blood of patients with NASH, NAFLD, or obesity. BhattacharjeeJ[23] found increased numbers of NKT cells in the livers of NASH mice. In our experiment, it was also observed that the proportion of NKT cells in the livers of NASH mice, and the levels of related inflammatory factors such as IFNγ were significantly increased.

T cell-mediated adaptive immunity can cause inflammation and fibrosis in NASH through cytotoxicity, cytokines, and other proinflammatory and profibrotic mediators[24]. CD3 is a surface marker for mature T lymphocytes. T cells are divided into two categories, ɑβ T cells (such as CD4 and CD8) and γδ T cells, according to different TCRs[25]. This study showed that, compared with the normal group, the proportion of CD3 + T cells in the HFHCC group was significantly higher than that in the control group, while no significant difference was found in CD4 + T cells and CD8 + T cells. IL5, IL13, and IL17A are mainly secreted by adaptive immune cells, such as Th2, Th12, and CD4 + T cells, and these cytokines did not change significantly after modeling. Moreover, there were no significant differences in the number of B cells. Overall, it is hypothesized that the HFHCC diet has a weaker effect on adaptive immunity than on innate immunity. However, a deeper understanding of the mechanism requires further verification and research.

This study had some potential limitations. Inconsistent with the disease characteristics of most patients with NASH, the weight of the model mice was lower than that of the normal group, which was mainly related to the involvement of bile salts in bile acid metabolism affecting energy metabolism; on the other hand, this model also provided a reference for the study of non-obese NASH. In addition, due to the limitation of the number of experimental channels for flow analysis, the study only used immunohistochemistry for the analysis of liver macrophages, and lacked the results of flow analysis; in
terms of adaptive immunity, Th1, Th2, Th17, and Treg have not been further detected and analyzed, which is also the direction for further research in the later stage.

In conclusion, we established an HFHCC diet-induced NASH model that is stable and reproducible, showing the emergence of NASH pathological features such as inflammation, steatosis, and fibrosis. This model is likely to trigger innate immunity. This could serve as a suitable experimental model for drug testing and for understanding the pathogenesis of innate immunity in NASH.

**Abbreviations**

NAFLD: Nonalcoholic fatty liver disease

NASH: Nonalcoholic steatohepatitis

HFHCC: high-trans fat, high-carbohydrate, and high-cholesterol diet

TG: Triglyceride

TC: Total cholesterol

LDL-C: low-density lipoprotein cholesterol

HDL-C: high-density lipoprotein cholesterol FBG: Fasting blood glucose

ALT: Alanine aminotransferase

AST: Aspartate transaminase

GLU: glucose

FBG: The fasting blood-glucose

H&E: hematoxylin and eosin

IHC: Immunohistochemical

Col-1: collagen type 1

α-SMA: α-smooth muscle actin

KCs: Kupffer cells

DCs: dendritic cells

TNF-α: Tumor necrosis factor-α

IFN-γ: γ-Interferon
NK cells: natural killer
NKT: natural killer T cells
MPO: myeloperoxidase
CCL: C-C motif chemokine
CXCL: C-X-C motif chemokine
G-CSF: macrophage colony stimulating factor
GM-CSF: granulocyte-macrophage colony stimulating factor

Declarations

Ethics Approval and Consent to Participate:
All experimental procedures were performed in accordance with the National Institutes of Health Guidelines for Laboratory Animals and approved by the Animal Ethics Committee of Shanghai University of Chinese Medicine (Permission Number: 201710017).

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Author Contributions:
QF, YJL, YYH designed this study. QZ, YJ, XX, ZMA and XW performed the experiments. QZ, YJ, XX analyzed the data. QZ wrote the manuscript. QF, YJL revised the manuscript. All authors critically participated in the discussion and commented on the manuscript. Qian Zhang, Yue Jin and Xin Xin contributed equally to this work.

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Data Availability Statement:
The datasets used and/or analyzed during the current study are available from the corresponding author upon request.

Conflicts of Interest:
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References


Table

Table 1 is available in the Supplementary Files section.

Figures
Figure 1

HFHCC Induced hepatic steatosis, inflammation and fibrosis in mice. (A). H&E (40x magnification) and SR staining (40x magnification) on liver sections, col-1, α-SMA immunohistochemical staining (200x magnification) (B). Hepatic TG content. (C). Sirius red collagen staining positive area (D). Fibrosis stage score (E). NAS score, (F). Hepatic ALT contents, (G). Hepatic AST contents of the mice in each group. *P < 0.05, **P < 0.01
Figure 2

HFHCC Induced changes in innate immune state in mice (A). macrophage F4/80 and Neutrophil MPO immunohistochemical staining results on liver tissue. (B). The proportion of NKT cells, NKT cells, DC cells in mouse liver. *P < 0.05, **P < 0.01
Figure 3

HFHCC Induced changes in adaptive immune state in mice (A). The proportion of B cells, CD3⁺T cells, CD4⁺T cells, CD8⁺T cells. *P < 0.05, **P < 0.01
Figure 4

HFHCC Induced changes in the proportion of hepatic cytokine levels. (A). Heatmap of serum inflammatory cytokines. Rows represent cytokine species and columns represent sample groups. (B). Interleukin-like pro-inflammatory cytokines IL-1α, IL-1β, IL-2, IL-6, IL-9, IL-12 (P40), IL12 (P70), IL-17A, Interleukin-like anti-inflammatory cytokines IL-4, IL-5, IL-10, IL-13 protein levels.*P < 0.05, **P < 0.01. (C). The protein levels of tumor necrosis factor TNF-α and interferon-like inflammatory factor IFN-γ. (D). The protein levels of chemokines CCL2, CCL3, CCL4, CCL5, CCL11, CXCL2. (E) The protein levels of colony stimulating factor GM-CSF,G-CSF,IL-3. *P < 0.05, **P < 0.01

Supplementary Files

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