IFN-γ deficiency attenuates hepatic inflammation and fibrosis in a steatohepatitis model induced by a methionine- and choline-deficient high-fat diet

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Luo X, Takahara T, Kawai K, Fujino M, Sugiyama T, Tsuneyama K, Tsukada K, Nakae S, Zhong L, Li X. IFN-γ deficiency attenuates hepatic inflammation and fibrosis in a steatohepatitis model induced by a methionine- and choline-deficient high-fat diet. Am J Physiol Gastrointest Liver Physiol 305: G891–G899, 2013. First published October 17, 2013; doi:10.1152/ajpgi.00193.2013.—Cytokines play important roles in all stages of steatohepatitis, including hepatocyte injury, the inflammatory response, and the altered function of sinusoidal cells. This study examined the involvement of a major inflammatory cytokine, interferon-γ (IFN-γ), in the progression of steatohepatitis. In a steatohepatitis model by feeding a methionine- and choline-deficient high-fat (MCDHF) diet to both wild-type and IFN-γ-deficient mice, the liver histology, expression of genes encoding inflammatory cytokines, and fibrosis-related markers were examined. To analyze the effects of IFN-γ on Kupffer cells in vitro, we examined the tumor necrosis factor-α (TNF-α) production by a mouse macrophage cell line. Forty two days of MCDHF diet resulted in weight loss, elevated aminotransferases, liver steatosis, and inflammation in wild-type mice. However, the IFN-γ-deficient mice exhibited less extensive changes. RT-PCR revealed that the expression of tumor necrosis factor-α (TNF-α), transforming growth factor-β, inducible nitric oxide synthase, interleukin-4 and osteopontin were increased in wild-type mice, whereas they were significantly suppressed in IFN-γ-deficient mice. Additionally, in vitro experiments showed that, when RAW 264.7 macrophages were treated with IFN-γ, they produced TNF-α in a dose-dependent manner. The present study showed that IFN-γ deficiency might inhibit the inflammatory response of macrophages cells and subsequently suppress stellate cell activation and liver fibrosis. These findings highlight the critical role of IFN-γ in the progression of steatohepatitis.

Steatohepatitis is one of the leading causes of liver-related morbidity and mortality in developed Western countries. The features of steatohepatitis, regardless of whether it is nonalcoholic (NASH) or alcoholic steatohepatitis include steatosis, liver cellular damage, inflammation, and varying degrees of fibrosis (48, 52, 70). Although the exact mechanisms that cause simple liver steatosis to progress to steatohepatitis remain poorly understood, the “two-hit” hypothesis is the most commonly accepted model explaining such progression. Steatosis in this model represents the “first hit” that sensitizes cells vulnerable to subsequent stress. The “second hit” can include oxidative stress, recruitment of inflammatory cells, and dysregulated cytokine/adipokine production, which all synergistically lead to hepatocyte death by apoptosis or necrosis, and subsequent liver inflammation and fibrosis. In the past, it was generally believed that inflammation is followed by the development of hepatic steatosis (69). Cytokines have been shown to be central mediators of inflammation in steatohepatitis (15, 39, 46, 53). The effect of tumor necrosis factor-α (TNF-α) on the pathogenesis of liver steatohepatitis has been investigated in many studies, and it has been clearly demonstrated that the liver and adipose tissue TNF-α and TNF receptor 1 transcripts, as well as the serum TNF-α levels, were increased in patients with steatohepatitis (4). However, the role of interferon-γ (IFN-γ), a critical and pleiotropic cytokine, in the development of liver steatohepatitis is not yet clearly understood.

The previous studies mainly focused on examining the contribution of IFN-γ to acute liver and intestinal injuries in animal models, revealing that IFN-γ was pivotal in aggravating the acute liver injury induced by concanavalin A or lipopolysaccharide (10, 72) and that it played a central role in the intestinal inflammation induced by interleukins (11). In most experiments, IFN-γ production is generally considered to antagonize the development of liver fibrosis. For example, IFN-γ-deficient mice are more susceptible to liver fibrosis induced by carbon tetrachloride (CCL4) (59), and the antifibrogenic effect of IFN-γ is believed to be mediated via inhibiting hepatic stellate cell (HSC) activation and TGF-β signaling (6, 51, 56, 57, 73). In contrast, in mice fed the MCD diet-induced steatohepatitis, Yu et al. identified significantly increased proinflammatory cytokines, including IFN-γ, CXCL1, CXCL10, and CCL3 (80). Furthermore, fatty liver in mice fed on hypercaloric or choline-deficient diets promotes IFN-γ production (39, 46). IFN-γ is pivotal for efficient innate and adaptive immune responses, and a detrimental role of IFN-γ in the initiation and/or maintenance of proinflammatory activation in development of obesity-associated insulin resistance and steatohepatitis has been reported (39, 46, 53). In addition, natural killer (NK) cells can kill...
activated HSC but not quiescent HSC, and activation of NK cells induced HSC death and ameliorated liver fibrosis in a mouse model of liver fibrosis. This effect was attributed to IFN-γ (55). However, these studies were performed by CCl4 or dimethylnitrosamine injection, or 3,5-diethoxycarbonyl-1,4-dihydrocollidine diet. The mechanisms of induction of fibrosis by those models are different from steatohepatitis. There have also been very few studies of the effects of IFN-γ on a liver fibrosis model induced by a methionine- and choline-deficient high-fat (MCDHF) diet, one of the most efficient models of induced steatohepatitis in rodents, and approved by the Committee on the Care and Use of Laboratory Animals at the National Research Institute for Child Health and Development.

**MATERIALS AND METHODS**

**Animal models.** IFN-γ-deficient mice on the C57BL/6J background were generated as described previously (64). Wild-type C57BL/6 mice were purchased from Shizuoka Laboratory Animal Center (Shizuoka, Japan). Ten-week-old male mice were housed four per cage in temperature- and light-controlled chambers for all experiments. Steatohepatitis was induced by feeding mice a MCDHF diet containing corn oil and sucrose [40% (wt/wt) fat and 40% (wt/wt) carbohydrates] for 42 days. Liver fibrosis was induced by feeding mice the MCDHF diet for 70 days. The liver was excised and divided into several parts for hematoxylin-eosin (HE) staining, immunostaining examination, and RNA extraction. All animal experiments were reviewed and approved by the Committee on the Care and Use of Laboratory Animals at the National Research Institute for Child Health and Development.

**Histopathological examination.** Liver tissue samples were kept in 10% formalin solution. Paraffin blocks were prepared as 4-μm cross sections, and HE staining and Sirius red staining were performed. The fibrotic areas were measured in three sections per mouse using an image analyzing system (VH analyzer; KEYENCE, Osaka, Japan).

**Serum biochemical detection.** Whole blood was collected, and serum was then evaluated for alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides, and cholesterol, expressed as units per liter or milligram per deciliter, respectively.

**RNA preparation and quantitative reverse transcriptase-polymerase chain reaction.** The total RNA was extracted from frozen liver tissue using ISOGEN (Nippon Gene, Tokyo, Japan). Each 800-ng RNA sample was reverse-transcribed to cDNA using oligo(dT) primers and Super Script reverse transcriptase (Invitrogen, Life Technologies Japan) according to the manufacturer’s protocol. The target-specific primers and probes were designed on the basis of the reported cDNA sequences and were synthesized by Biotechonologies (Novato, CA). Quantitative RT-PCR was performed using the TaqMan system on the Applied Biosystem PRISM7700 instrument (Life Technologies Japan). Quantitative RT-PCR was conducted in 0.9 mM each primer in a 25-μl final reaction volume of Premix Ex Taq (Takara Bio, Shiga, Japan). The PCR cycling conditions were as follows: 50°C for 2 min, 95°C for 15 min and 50 cycles of 95°C for 30 s, 60°C for 1 min, and 25°C for 2 min. The data were expressed as the comparative cycle threshold (Ct) values. The normalized Ct value of each gene was obtained by subtracting the Ct value of 18s rRNA from the comparative cycle threshold (Ct) values. The normalized Ct value was analyzed by an ELISA kit (R&D Systems, Minneapolis, MN).

**Statistical analysis.** The data are presented as the means ± SE and were analyzed statistically using a one-way ANOVA, followed by Fisher’s protected least-significance difference test or the Mann-Whitney U-test. Values of P < 0.05 were considered to be statistically significant.

**RESULTS**

**IFN-γ deficiency attenuated the loss of body weight induced by MCDHF.** Initially, the average body weight was not significantly different between the wild-type and IFN-γ-deficient mice, but, after the mice had consumed the MCDHF diet for 42 days, they showed significant differences. The wild-type mice decreased in weight by 9.9 g on average, about 38% of their initial body weight, whereas the weight of the IFN-γ-deficient mice decreased by 7.0 g on average, about 28% of their initial body weight (Fig. 1A).

**IFN-γ deficiency reduced the MCDHF-induced liver inflammation.** As in our previous study, the MCDHF diet induced liver steatosis (40, 41). In this study, fatty droplets formed in both wild-type and IFN-γ-deficient mice, inducing macrovesicular and microvesicular steatosis after 42 days of the MCDHF diet. The quantitative analysis of the percentage of steatotic area was similar in both groups (data not shown), whereas HE staining revealed a different degree of inflammatory cell infiltration between the two groups. In wild-type mice, the clusters of inflammatory cells and enlarged form cells were clear and prevalent, which were scarcely seen in IFN-γ-deficient mice (Fig. 1B). Consistent with the HE staining, F4/80 immunostaining revealed a marked increase in the number of activated and infiltrated Kupffer/macrophage cells in wild-type mice, which were localized at the enlarged cells detected as form cells; however, these were considerably reduced in IFN-γ-deficient mice (Fig. 1C). We further analyzed the surface markers of macrophages, and immunostaining for CD11c showed that the enlarged form cells and small cells around the form cells were strongly positive for CD11c in wild-type mice, whereas this was seldom seen in IFN-γ-deficient mice (Fig. 1D).

Because macrophages are the primary source of inflammatory cytokines in this steatohepatitis model, we analyzed the liver tissue mRNA expression levels of several inflammatory cytokines, including TNF-α, interleukin (IL)-4, transforming growth factor (TGF)-β1, inducible nitric oxide synthase, osteopontin (OPN), and IFN-γ. In wild-type mice, the IFN-γ levels were significantly increased after 42 days of the MCDHF...
diet, whereas the level was undetectable in IFN-γ-deficient mice at all of the time points examined. The fact that IFN-γ/H9253 was not able to be detected in IFN-γ/H9253-deficient mice demonstrated the accuracy of the gene knockout animal used in this study. The levels of other inflammatory cytokines were also obviously increased after 42 days of the MCDHF diet in wild-type mice. Of note, the gene expression of these inflammatory cytokines was significantly suppressed in IFN-γ/H9253-deficient mice fed the MCDHF diet (Fig. 1).

**Fig. 1.** The body weight loss and inflammatory cell infiltration were attenuated in interferon-γ (IFN-γ)-deficient mice fed the methionine- and choline-deficient high-fat (MCDHF) diet for 42 days. A: the body weight loss in wild-type and IFN-γ-deficient groups. B: large fatty droplets, form cells (red arrows), and the infiltration of inflammatory cells (black arrows) in wild-type mice. Fatty droplets were similarly present, but inflammatory cells were significantly decreased, in IFN-γ-deficient mice. C: numerous crowded clusters of F4/80-positive macrophages were recruited in wild-type mice (arrows), which were significantly reduced in IFN-γ-deficient mice. The results of quantification of F4/80-positive macrophages analyzed using an image analyzing system (VH analyzer; KEYENCE). Scale bars represent 100 μm (means ± SE; **P < 0.01 vs. wild-type mice). D: CD11c was weakly detected in the form cells in the wild-type mice (arrows), where F4/80 was coimmunolocalized, whereas CD11c was significantly reduced in IFN-γ-deficient mice. Small cells around the form cells were clearly positive for CD11c in the wild-type mice (arrowheads). Scale bars represent 100 μm. E: the expression of inflammatory cytokine genes was downregulated in IFN-γ-deficient mice after they were fed the MCDHF diet for 42 days. TNF-α, tumor necrosis factor-α; IL-4, interleukin-4; TGF-β, transforming growth factor-β; iNOS, inducible nitric oxide synthase; OPN, osteopontin. The data are representative of 4–5 independent experiments and indicate the mean ratio of triplicate results from each experiment (arbitrary/unit, means ± SE; *P < 0.05 and **P < 0.01 vs. wild-type mice).
both wild-type and IFN-γ-deficient mice that had not fasted, the MCDHF diet induced decreased serum triglyceride and cholesterol levels; however, the change in the cholesterol levels in wild-type mice was slightly larger (Fig. 2B).

IFN-γ deficiency reduced the MCDHF-induced liver fibrosis. Sirius red staining showed a moderate perisinusoidal collagen deposition starting from the central area and extending into the hepatic lobules, which represented 13% of the liver area in wild-type mice after 70 days of MCDHF diet (Fig. 3A). This amount of collagen was effectively reduced in IFN-γ-deficient mice fed the MCDHF diet, accounting for 3% of the liver area (Fig. 3A), which demonstrated that the collagen deposition was largely suppressed by IFN-γ deficiency.

In the α-SMA immunostaining, numerous α-SMA-positive cells were located around the central areas and infiltrated into the middle part of the lobules around the form cells after wild-type mice were fed the MCDHF diet for 70 days (Fig. 3B). However, there were very few α-SMA-positive cells, except in some vessel areas, in the IFN-γ-deficient mice after 70 days of the MCDHF diet (Fig. 3B). This indicated that activation of HSCs into α-SMA-positive myofibroblasts was largely prevented by IFN-γ deficiency. We also analyzed the liver tissue fibrosis-related gene expression levels, including those of α-SMA, type I collagen, tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), and matrix metalloproteinase-2 (MMP-2), which were obviously increased in wild-type mice whereas these did not occur, or were much less severe, in IFN-γ-deficient mice. Of interest, the mRNA levels of IFN-γ were increased in the liver tissue of wild-type mice fed the MCDHF diet (Fig. 1E). This result was supported by a study revealing that the IFN-γ expression was enhanced in steatohepatitis patients with hepatitis C virus infection (23). The increased expression of the IFN-γ levels in different types of steatohepatitis confirms its importance in steatohepatitis arising because of various pathogenic processes. In the present study, the levels of other inflammatory cytokines also simultaneously increased in the wild-type mice but were largely suppressed in IFN-γ-deficient mice (Fig. 1E). More importantly, this phenomenon was consistent with hepatic F4/80 immunostaining, a marker of macrophage/Kupffer cells, which showed an obvious increase in wild-type mice that was inhibited in IFN-γ-deficient mice fed the same MCDHF diet (Fig. 1C). In addition, CD11c, a marker of proinflammatory M1 macrophages, was also prominently expressed in wild-type mice and was significantly suppressed in IFN-γ-deficient mice (Fig. 1D). To further estimate the influence of IFN-γ on macrophages/Kupffer cells in vitro, we examined the TNF-α production by RAW 264.7 cells treated with different concentrations of IFN-γ in the culture medium, there was a dose-dependent increase in TNF-α production compared with the control group (Fig. 4).

**DISCUSSION**

In our study, the MCDHF diet induced serum biochemical, liver histological, and molecular changes in wild-type mice, whereas these did not occur, or were much less severe, in IFN-γ-deficient mice. To further evaluate the influence of IFN-γ on Kupffer cells in vitro, we examined the TNF-α production by a mouse macrophage cell line (RAW 264.7). We found that, when the RAW 264.7 cells were treated with different concentrations of IFN-γ, the TNF-α production was significantly increased in wild-type mice but was largely suppressed in IFN-γ-deficient mice (Fig. 4). This result was further confirmed by the decreased TNF-α production in IFN-γ-deficient mice after 70 days of the MCDHF diet (Fig. 1E). This result was supported by a study revealing that the IFN-γ expression was enhanced in steatohepatitis patients with hepatitis C virus infection (23). The increased expression of the IFN-γ levels in different types of steatohepatitis confirms its importance in steatohepatitis arising because of various pathogenic processes. In the present study, the levels of other inflammatory cytokines also simultaneously increased in the wild-type mice but were largely suppressed in IFN-γ-deficient mice (Fig. 1E). More importantly, this phenomenon was consistent with hepatic F4/80 immunostaining, a marker of macrophage/Kupffer cells, which showed an obvious increase in wild-type mice that was inhibited in IFN-γ-deficient mice fed the same MCDHF diet (Fig. 1C). In addition, CD11c, a marker of proinflammatory M1 macrophages, was also prominently expressed in wild-type mice and was significantly suppressed in IFN-γ-deficient mice (Fig. 1D). To further estimate the influence of IFN-γ on macrophages/Kupffer cells in vitro, the TNF-α production by RAW 264.7 cells was con-

![Fig. 2](http://ajpgi.physiology.org/) The serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels (A) and triglyceride and cholesterol levels (B) in wild-type and IFN-γ-deficient mice fed the MCDHF diet for 42 days (means ± SE; *P < 0.05 and **P < 0.01 vs. wild-type mice).
firmed to dose-dependently increase by IFN-γ (Fig. 4), consistent with previous reports (18, 74). Considering the interconnection between resident Kupffer cells, infiltrated macrophages, and various cytokines, as well as their pivotal roles in the progression of steatohepatitis reported in previous studies (7, 12, 13, 26, 60, 71), we suggest that decreased macrophage recruitment or reduced Kupffer cells in the environment induced by IFN-γ deficiency could account for the decreased mRNA levels of the above inflammatory cytokines as described previously using a T cell-mediated hepatitis rodent model (31).

In our study, although IFN-γ appeared to contribute extensively to the inflammatory response, it did not appear to have a role in the initiation or aggravation in steatosis, as seen in the liver histology and serum biochemistry examinations. Interestingly, IL-6-deficient mice fed the MCDHF diet also did not show any major change in the progression of steatosis in their study (52). In the steatohepatitis model induced by MCDHF,
our findings therefore suggest that the development of liver steatosis is mainly the result of the diet itself, rather than IFN-γ or other inflammatory cytokines.

In a previous study, IFN-γ was demonstrated to be centrally involved in acute liver cell injury as assessed by the serum ALT and AST levels (29). In our present study, IFN-γ deficiency also effectively attenuated the MCDHF diet-induced augmentation of the ALT and AST levels (Fig. 2A). This was probably related to the fact that the decreased levels of cytokines such as TNF-α, a death factor for hepatocytes, lessened the damage to hepatocytes. Taken together, our data suggest that IFN-γ was not only involved in the liver inflammation but also in the hepatocyte injury throughout the progression of steatohepatitis. In fact, IFN-γ induces hepatocyte apoptosis (25), likely because inflammatory cytokines and cell injury are usually interconnected.

In human NASH, in general, patients gain body weight. Unlike with human NASH, murine NASH by MCDHF diet results in body weight loss (Fig. 1A). Similar findings were also reported by another group who demonstrated that IFN-γ-deficient mice showed an obviously attenuated weight loss compared with wild-type mice when they received the same intraperitoneal injections of IL-12 and IL-18 (11). We propose that IFN-γ deficiency is beneficial for protecting mice against the weight loss induced by MCDHF, even though some weight loss still occurred in the IFN-γ deficient mice.

In wild-type mice fed the MCDHF diet for 70 days, the Sirius red staining and α-SMA-immunopositive cells were significantly increased, and the liver mRNA expression levels of type I collagen, α-SMA, TIMP-1, and MMP-2 were also dramatically elevated, which demonstrated liver fibrosis. The increase of MMP-2 was likely the result of the inflammation of the liver, as we reported previously (66). Compared with wild-type mice, the Sirius red staining and α-SMA-positive cell immunostaining both decreased (Fig. 3, A and B), and the gene expression levels of type I collagen, α-SMA, TIMP-1, and MMP-2 also significantly decreased (Fig. 3C) in IFN-γ-deficient mice, which indicated a reduced liver collagen content and decreased HSC activity resulting from the MCDHF diet. These results provided apparent evidence of an IFN-γ dependence of the liver fibrosis. Interestingly, IFN-γ has been shown in numerous previous studies to be antifibrogenic, by both inhibiting the activation of HSCs in culture as well as attenuating fibrosis in models such as the CCl4 and dimethyl-nitrosamine, and IFN-γ treatment ameliorated liver fibrosis (56). IFN-γ displays antifibrotic effects in HSCs via the impairment of TGF-β signaling (77), the inhibition of collagen production (8), and the suppression of SMA expression (56, 58). Furthermore, IFN-γ activation of signal transducer and activator of transcription 1 is effective in ameliorating liver fibrosis in animal models, and IFN-γ treatment improves the fibrosis scores in patients with chronic hepatitis B virus infection (78, 79). In addition, IFN-γ inhibits extracellular matrix/collagen expression in stellate cells by virtue of a number of effects on stellate cells (5, 6, 27, 28, 57). On the other hand, IFN-γ is a very potent proinflammatory cytokine with a ubiquitous receptor expression, and therefore IFN-γ-based experimental therapies are associated with side effects like severe flu-like symptoms, systemic endothelial and immune cell activation, neurotropic effects, and hyperlipidemia (36, 54). IFN-γ also works in accumulation of neutrophils and macrophages in the liver (42) and is known to be a key molecule in the induction of type I polarization (34, 43). Given that, in the present study, the antifibrotic effect by IFN-γ attenuation might be because of the reduction of inflammation and not a direct effect of IFN-γ to HSCs. In concordance, caspase-1, an important component of inflammation, knockout mice on the MCD diet showed marked reduction in mRNA expression of genes involved in inflammation and fibrogenesis with significant reduction of hepatic collagen deposition (14). In contrast, some studies reported a different result. For example, IFN-γ therapy was not able to attenuate or reverse liver fibrosis in a double-blind clinical trial including 502 patients (54), and IFN-γ itself promoted the hepatic progenitor cell response and exacerbated fibrosis in a chronic liver injury model (38). Considering the unexpected result of attenuated liver fibrosis resulting from IFN-γ deficiency in our present study, we made the following assumptions. There might have been a prior event that ameliorated the liver injury and inflammation induced by decreasing the macrophages/Kupffer cell infiltration and suppressing the inflammatory response seen in IFN-γ-deficient mice, and this would probably underlie the attenuated liver fibrosis. A number of previous studies demonstrated that the severity of liver fibrosis was closely related to inflammatory cytokines, because they triggered stellate cell activation into α-SMA-positive myofibroblasts, orchestrating a cross talk between different cell types and different stages of steatohepatitis (17, 71). In fact, IFN-γ induces the accumulation of neutrophils and macrophages in the liver (42) and also induces type I polarization (34, 43). Hepatic accumulation of inflammatory cells is generally greater in NASH than in steatosis, suggesting that activation of the immune system may contribute to progression of fatty liver damage. The liver harbors resident populations of cells that regulate innate immune responses (22). The mRNA expression and histological analysis revealed significantly higher expression of IFN-γ and cellular infiltration in liver by MCHD diet (Fig. 1, B and E). These data suggested the possibility that the infiltrated cells might express abundant IFN-γ. Several cell populations have been known to express IFN-γ, in not only CD4+ T cells, CD8+ T cells, γδ T
cells, natural killer T (NKT) cells, and NK cells but also in macrophages, dendritic cells, and B cells (20). For instance, NF-κB1 deficiency stimulates the progression of NASH in association with the MCD diet in mice by promoting NKT cell-mediated responses with an upregulation in the production of IFN-γ and OPN (47). Additionally, many previous studies have suggested that the accumulation of infiltrated NKT cells in the liver is involved in pathogenesis of steatohepatitis, and IFN-γ may involve it (1, 30, 47, 62, 63, 65).

In addition to NKT cells, a previous study on a pediatric NASH patient described that the hepatic microenvironment is dominated by IFN-γ but not IL-4, and it is infiltrated by a higher number of CD8+ cells. The number of infiltrating neutrophils positively correlated with reactive oxygen species generation by peripheral polymorphonuclear cells. A distinctive increase in CD8+ CD45RO and CD8+ CD45RA subpopulations and an increased production of IFN-γ by CD4+ and CD8+ cells was also demonstrated (16). The patients with NASH demonstrated a significantly higher ratio of IFN-γ+/CD4 T cells in the liver (32). A NASH murine model that was fed atherogenic high-fat diet demonstrated an increased CD8+/CD4+ T cell ratio that is also comparable with the clinical NASH patient pathology (61). Helper T cell activation, which induces the production of Th1 cytokines, is thus considered to be a pathogenic finding in steatohepatitis (16, 53). In addition, in patients with NASH, the ratio of neutrophils to lymphocytes increases (2), suggesting that granulocytes are involved in the pathogenesis of NASH. Furthermore, many of the recent data showed that innate immune processes both within and outside the liver are involved in NASH (50, 81). Both the previous studies and our findings suggest that a decrease in the IFN-γ levels might therefore inhibit the infiltration of inflammatory cells.

Among these cytokines, TNF-α signaling is considered to be especially important for liver fibrosis (3, 21, 71, 75), and TGF-β1 plays a critical role in liver fibrosis (9, 19, 33). Furthermore, OPN, which is an activator of HSC (62, 63) that induces the production of Th1 cytokines, is thus considered to be a pathogenic finding in steatohepatitis (16, 53). In addition, in patients with NASH, the ratio of neutrophils to lymphocytes increases (2), suggesting that granulocytes are involved in the pathogenesis of NASH. Furthermore, many of the recent data showed that innate immune processes both within and outside the liver are involved in NASH (50, 81). Both the previous studies and our findings suggest that a decrease in the IFN-γ levels might therefore inhibit the infiltration of inflammatory cells.

No conflicts of interest, financial or otherwise, are declared by the authors.

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