Cytokine 124 (2019) 154519

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

The role of IFN in the development of NAFLD and NASH

Michelle Møhlenberg[a], Ewa Terczynska-Dyla[a], Karen Louise Thomsen[b], Jacob George[c], Mohammed Eslam[c], Henning Grønbæk[b], Rune Hartmann[a,*]

[a] Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark
[b] Department of Hepatology and Gastroenterology, Aarhus University Hospital, Aarhus, Denmark
[c] Storr Liver Centre, The Westmead Institute for Medical Research, University of Sydney and Westmead Hospital, Westmead, Australia

ARTICLE INFO

Keywords:
Non-alcoholic fatty liver disease (NAFLD)
Non-alcoholic steatohepatitis (NASH)
Interferons (IFN) type I IFN and type III IFN
Humans and human genetics
Animal models

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) and its progressive inflammatory form non-alcoholic steatohepatitis (NASH) are major health challenges due to a significant increase in their incidence and prevalence. While NAFLD is largely benign, the chronic liver inflammation in NASH patients may cause progression to liver cirrhosis and hepatocellular carcinoma. There is an urgent need for a better understanding of the factors, which drive the progression from NAFLD to NASH and how to use this information both to improve diagnostic and to develop new treatment strategies. Increasing evidence points to interferons (IFNs) as key players in NAFLD and particular in the progression to NASH. IFNs crucial role in disease development is supported by both genetic evidence and animal studies. In this review, we describe the involvement of both type I and type III IFNs in the development and progression of NAFLD and NASH.

1. The epidemiology and pathophysiology of NAFLD

Non-alcoholic fatty liver disease (NAFLD) is a global epidemic estimated to affect 20–30% of the general population [1]. Not surprisingly, NAFLD is the leading cause of chronic liver disease in the Western world [2] and therefore also among the leading causes of liver transplantation in North America [3].

The spectrum of NAFLD ranges from simple steatosis, i.e. the accumulation of lipids in hepatocytes, to the inflammatory form of the disease, steatohepatitis (NASH), which can progress to liver fibrosis and cirrhosis. This transition is a cardinal feature of progressive disease, which in up to 20% of NASH patients will result in development of liver cirrhosis [4], and it is also a major risk factor for the development of hepatocellular carcinoma [5,6]. The specific molecular mechanisms driving this transition and chronic inflammation in NASH patients are currently unknown, but innate immune signalling plays a pivotal role.

The pathogenesis of NASH is complex and was initially described by a “two-hit” hypothesis where the “first hit” led to hepatic steatosis and was followed by a “second hit”, leading to the development of steatohepatitis and fibrosis [7]. This model is superseded by a “multiple parallel hits” hypothesis in which a number of different processes contribute to hepatic steatosis, inflammation and fibrosis contemporaneously rather than sequentially. Inflammatory mediators, especially those deriving from the gut-liver axis, infiltrating immune cells and the adipose tissue, all play crucial roles in making NAFLD/NASH a systemic metabolic disorder [8].

2. Metabolic and genetic risk factors associated with development of NAFLD and transition to NASH

NAFLD is a complex phenotype with substantial inter-individual variation in disease progression. Hence, a dynamic interaction between the environment and a polygenic background determine disease susceptibility, progression and outcomes [9]. Risk factors for NAFLD include obesity [10,11], type 2 diabetes mellitus [12–14], dyslipidemia, hypertriglyceridemia [15], a history of cyclic weight gain and loss [16], hypertension [17] and alterations in gut microbiota [18]. Recent work has revealed several genetic modifiers of NAFLD: The palatin-like phospholipase domain-containing 3 (*PNPLA3*) gene is consistently identified as a major common genetic determinant of the entire spectrum of NAFLD [19,20]. Other characterised variants, which influence the development of disease, include the transmembrane 6 superfamily member 2 (*TM6SF2*) [21,22], glucokinase regulator (*GCKR*) [23] and hydroxysteroid (17β) dehydrogenase 13 (*HSD17B13*) [24] genes. Finally, a variant in the membrane bound O-acyltransferase domain-containing 7 (*MBOAT7*) gene, originally discovered as a risk variant for alcoholic cirrhosis [25], is also a risk variant for NAFLD progression [26] and even for viral liver diseases [27,28].

* Corresponding author.
E-mail address: rh@mbg.au.dk (R. Hartmann).

https://doi.org/10.1016/j.cyto.2018.08.013
Received 15 June 2018; Received in revised form 10 August 2018; Accepted 11 August 2018
Available online 21 August 2018

Inflammation and fibrosis share common pathways across different liver disease aetiologies. Therefore, it was not surprising that several single nucleotide polymorphisms (SNPs) located within the interferon (IFN) lambda (*IFNL3/IFNL4*) region and originally described as a genetic determinant of hepatitis C virus (HCV) clearance, have been linked to the progression from NAFLD to NASH [29–31]. This discovery underlines the potential role of the IFNs in the development of NASH but also highlights our lack of a detailed mechanistic understanding of how IFNs influence hepatic inflammation and disease. This we will discuss in more detail in the following sections.

3. Interferons (IFNs)

The IFNs are classic antiviral cytokines, which constitute our crucial first line of defence against infections, and they are divided into three groups (type I, II and III IFNs) based on their receptor usage. Type II IFN (IFN-γ) originates largely from T-helper cells and is mainly associated with the adaptive immune system and Th1 type immune responses and is therefore mechanistically completely different from the classical antiviral IFNs (type I and type III IFNs), which are the focus of this review. Hence, type II IFN is not considered further.

The IFNs are produced and secreted as a response to pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), which are detected by a variety of pattern recognition receptors (PRRs) on host cells. These PRRs activate the transcription factors, primarily nuclear factor κB (NFκB) and IFN regulatory factors (IRFs), which subsequently drive transcription of *IFN* genes (Fig. 1, left).

Almost all nucleated cells respond to type I IFNs (IFN-α and IFN-β), which therefore mediate a strong systemic response, sometimes at the cost of significant immune pathology [32]. In contrast, the spectrum of action for the type III IFNs (IFN-λ) is much more limited, their primary role appears to provide an efficient first line defence of mucosal surfaces [33]. Human hepatocytes respond well to IFN-λ [34,35] and pegylated-IFN-λ1 was effective in treating HCV in a phase II clinical trial [36], suggesting that human hepatocytes respond well to IFN-λ *in vivo*. In contrast, mice hepatocytes showed low or no response both *in vitro* and *in vivo* [37]. Type I and type III IFNs signal via two different heterodimeric complexes, nevertheless they both lead to activation of highly similar subset of IFN stimulated genes (ISGs) [38]. The engagement of both receptor complexes leads to a phosphorylation cascade, which triggers the activation of signal transducer and activator of transcription proteins (STATs). Type I and type III IFNs have a unique ability to activate the transcription factor STAT2 and signalling leads to the formation of a STAT1/STAT2 heterodimer, which interacts with IFN regulatory factor 9 (IRF9) to form the IFN-stimulated gene factor 3 (ISGF3) complex. The ISGF3 complex translocates to the nucleus where it binds IFN-stimulated response elements (ISRE) and activates the transcription of hundreds of ISGs (Fig. 1, right).

4. Genetic variation within the *IFNL3/4* locus and liver diseases

Humans possess four functional type III IFN genes, designated *IFNL1* to *-4,* which are clustered together on chromosome 19 (19q13.13-2). The *IFNL3* and *IFNL4* genes are transcribed in the opposite direction of the *IFNL1* and *IFNL2* genes (Fig. 2A). In other mammals, the *IFNL* genes are organized and transcribed in similar directions [39], although not all four genes are present in every species. Notably, the *IFNL4* gene is not present in mouse and rat genomes. A phylogenetic analysis of different mammals revealed that IFN-λ4 constitutes a separate clade in the type III IFN tree, whereas IFN-λ1, -2 and -3 group according to species [33]. This suggests that the common ancestor of all mammals had two different *IFNL* genes, one *IFNL1-3*-like and one *IFNL4*-like. The *IFNL1-3*-like gene has undergone several independent duplication events during the evolution, giving rise to the *IFNL1-3* genes, whereas the *IFNL4* gene is evolutionarily conserved in mammals, indicating its functional relevance [40]. However, in the majority of the human population, *IFNL4* is a pseudogene due to a substitution of a single guanine base to two thymines (rs368234815 TT/ΔG) [41]. The ancestral ΔG variant of the rs368234815 SNP creates a functional IFN-λ4 protein, whereas the TT insertion causes a frameshift and thus eliminates its expression. The rs368234815 SNP is in high linkage disequilibrium with the rs12979860 SNP [41] and another SNP (rs4803217) in the 3′UTR region of *IFNL3* [42]. Originally, the genetic variations within the *IFNL3/IFNL4* region were linked to the outcome of HCV treatment as well as spontaneous clearance of HCV [43,44]. These variations create three

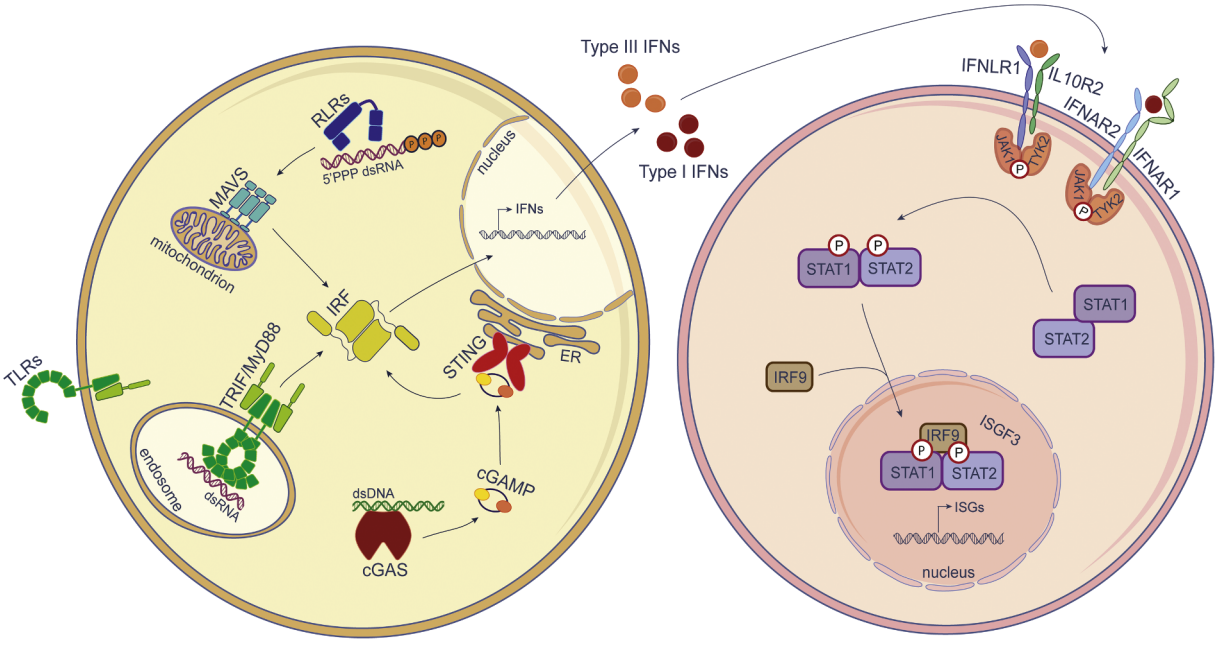
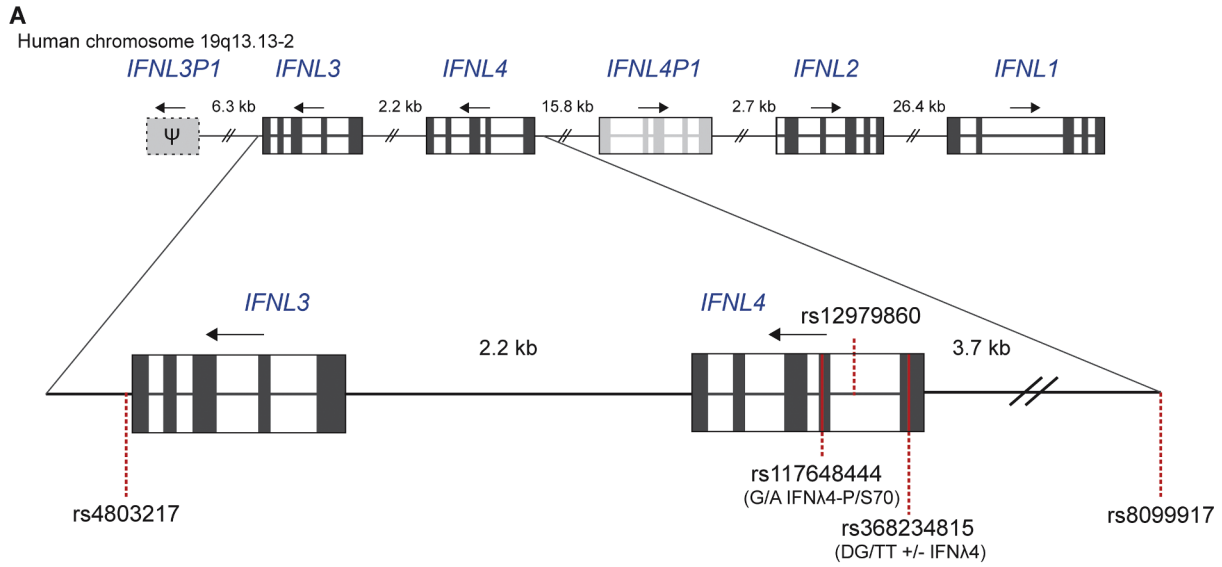


Fig. 1. Type I and type III interferon induction and signalling. Activation of pattern recognition receptor pathways lead to activation of interferon response factors (IRFs), which induce interferon (IFN) production. The IFNs are secreted out of the cell and signalling occurs through a heterodimeric receptor complex distinctive to the IFN type. The downstream signalling pathways are similar in type I and type III IFNs and lead to the induction of interferon stimulated genes (ISGs).



B

GWAS marker	Functional variant	GWAS marker	Functional variant	Functional variant	Phenotypes			
rs8099917	rs368234815	rs12979860	rs117648444	rs4803217	IFN-λ4 expression	IFN-λ4 activity	Viral clearance	Inflammation and fibrosis
T	TT	C	G	G	None	None	High	High
T	ΔG	T	A	T	IFN-λ4 S70	Moderate	Moderate	Low
G	ΔG	T	G	T	IFN-λ4 P70	High	Low	Low

Fig. 2. Schematic organisation of the *IFNL* genes and genetic variants. The genomic organization of the type III IFNs in humans (A). The functional genes are shown in dark grey, whereas pseudogenes are coloured light grey. All exons and introns are drawn in scale except for the pseudogene with the ψ symbol and the distances between the genes. Arrows indicate the direction of transcription. Functional variants and GWAS markers in the *IFNL3/IFNL4* genomic region grouped according to haplotype and shown with their associated phenotypes (B).

dominant haplotypes in Caucasians and Asians (see Fig. 2), which have significantly different clearance rates of the HCV infection [41,45]. The haplotypes are characterised by different activity levels of IFN-λ4. The first haplotype with a non-functional *IFNL4* gene is characterised by the TT allele for rs368234815 and allele C for rs12979860. The second haplotype encodes a highly active IFN-λ4-Pro70 and is characterised by the G allele of rs117648444 and the G allele for rs8099917 [41].The third haplotype with a lesser active IFN-λ4-Ser70 is characterised by allele A for rs117648444 [46]. (Fig. 2B).

5. The *IFNL3/IFNL4* genotype is associated with chronic liver inflammation and NAFLD

The *IFNL3/IFNL4* genotype not only affects HCV clearance but also demonstrates an effect on hepatic inflammation and fibrosis in HCV infected patients. Thus, patients harbouring SNPs associated with elimination of functional *IFNL4* (rs12979860 C or rs368234815 TT) exhibited better HCV clearance but also developed increased liver inflammation and fibrosis [47,48]. Importantly, it is now evident that the *IFNL3/IFNL4* genotype not only affects HCV-related liver outcomes but also has a broader aetiology-independent effect on the inflammatory state in the liver. In a study comprising a large cohort of patients with NAFLD or chronic hepatitis B or C, the rs12979860 C/C genotype was associated with increased hepatic inflammation and fibrosis in an aetiology-independent manner [31]. This result is in accordance with an independent study on a large cohort of NAFLD patients (n = 946) [29,30]. Interestingly, whether HCV-infected patients harboured the rs117648444 G allele (highly active IFN-λ4-Pro70) or the rs117648444 A allele (less active IFN-λ4-Ser70) did not appear to influence their degree of liver fibrosis [49]. This important observation opens up for two interpretations. Either *IFNL4* is not the causative gene in relation to liver fibrosis and the variants observed alter the transcription of the neighbouring *IFNL3* gene or the rs117648444 A allele is an adaptive mutation, which combines high viral clearance with a low degree of inflammatory damage. Further studies will be required to explain the effect of *IFNL3/IFNL4* SNPs on NAFLD.

Though the functional mechanisms of these genetic associations are not clear, some hypotheses can be postulated. Notably and similar to what has been reported in HCV patients [50], Petta et al. found that hepatic ISGs expression was increased in NAFLD patients carrying the functional *IFNL4* gene. [30]. This observation is different from a previous study showing that ISG expression differed according to *IFNL3/IFNL4* genotype in patients with HCV genotype 3, but not in patients with NAFLD [51]. The discrepancy between the two studies may be attributed to differences in sample size and ethnicity.

6. IFN involvement in the development and progression of NAFLD in mice models

Multiple animal studies are providing further evidence for a role for IFN in NAFLD. When fed a high-fat diet (HFD), mice lacking a functional type I IFN receptor (IFNαR1) are protected from steatosis and insulin resistance (IR) compared to wild-type (WT) mice [52]. This study observed the same protection in mice with IFNαR1-deficient bone marrow-derived $CD8^+$ T cells. Consistently, another study demonstrated that mice lacking IFNαR1 and deficient in plasmacytoid dendritic cells (pDCs) (B6.E2-2 fl/fl.Itgax-cre) fed with HFD were protected against metabolic syndrome and IR compared to WT mice [53]. However, IFNαR1-deficient mice are shown to be more susceptible to liver fibrosis than WT mice [54]. A time-course transcriptome study on a

choline-deficient (CD) diet NAFLD mice model, also observed that STAT1 and IFN-regulated genes were up-regulated compared to control, further suggesting a role for IFN signalling in NAFLD progression [55].

A recent study has investigated the tissue-specific role of type I IFN signalling in NAFLD, namely in hepatocytes, adipocytes, intestinal epithelial cells and myelocytes [56]. The study found a differential role for type I IFN signalling in each tissue affecting different aspects of NAFLD. Deleting IFNαR1 specifically in hepatocytes led to increased steatosis but no IR in methionine-/choline deficient diet (MCD) or HFD fed mice, whereas in adipocyte-IFNαR1-deficient mice increased IR and no steatosis was observed. In contrast, deletion of IFNαR1 in myeloid or intestinal epithelial cells did not affect susceptibility to metabolic or liver injury. The authors proposed that steatosis and IR are independent factors in NAFLD development and progression. However, it is also possible that the organ-specific knockout of IFNαR1 influences cells of the immune system and alters the disease. This might explain the aforementioned observation of protection from steatosis and IR in mice with IFNαR1-deficient bone marrow-derived $CD8^+$ T cells [52]. Taken together the IFNαR1 knockout studies clearly demonstrate the involvement of type I IFN in pathogenesis of NAFLD/NASH. However, whether a similar effect will be observed with type III IFN receptor knockout mice requires investigation.

7. IFN induction is involved in NAFLD progression

Recent reports suggest that IFN regulatory factors (IRFs), which are the key molecules involved in the induction of IFN transcription, play a pivotal role in NAFLD/NASH. Both IRF3 and IRF7 expression is increased in obese mice and humans [57,58]. Knockout of IRF3 protects from IR in HFD-fed mice [57] and IRF7-deficient mice show improved insulin sensitivity and glucose- and lipid-metabolism [58]. Consistently, higher expression of IRF5 was observed in human NASH compared to controls and IRF5 knockout in myeloid cells protected from hepatic fibrosis induced by metabolic or toxic stress in MCD and bile duct ligation (BDL) animal models [59]. Similarly, the expression of stimulator of IFN genes (STING), an important protein in the innate immune signalling pathway, and its downstream factor IRF3 were found to be upregulated in the livers of HFD-fed mice. Knocking down either STING or IRF3 led to an attenuation in free fatty acid (FFA)-induced hepatic inflammation and apoptosis [60], further suggesting a role for IFNs in the development of IR and inflammation. However, one study found IRF3 expression to be reduced in the liver of obese mice and if IRF3-deficient mice were given HFD they exhibited increased IR and steatosis [61]. The reasons for the discrepancy between studies is not clear but may be due to differences in study design or mice strain, suggesting that other factors such as microbiota might be involved. Furthermore, myeloid cell-specific IRF4 knockout mice on a HFD developed significant IR and increased expression of inflammatory genes [62], indicating IRF4 to be a negative regulator of inflammation in diet-induced obesity. Similarly, IRF9 was shown to protect against hepatic IR, steatosis and inflammation in whole-body IRF9 knockout mice [63].

Additionally, mitochondrial antiviral-signalling protein (MAVS) located upstream of IRFs in one of the IFN induction pathways (Fig. 1) is decreased in MCD-fed mice and NASH patients [64]. In conclusion, these data suggest that different IRFs may have differential impacts on NAFLD.

8. The role of toll-like receptors and the inflammasome in NAFLD

The gut-liver axis, including the recruitment of pro-inflammatory immune cells with release of inflammatory cytokines, e.g. IFNs, and adipose-tissue inflammation, plays a key role in the NAFLD pathogenesis (Table 1). A Western diet, for example, increases circulating levels of bacterial products including lipopolysaccharides (LPS) by altering gut flora and intestinal permeability in patients and in murine models [65,66]. Intra-hepatic sensing of these products by toll-like receptors (TLRs; particularly TLR2, -4 and -9) is a key event for the initiation of liver injury [67–69]. In particular, liver resident or infiltrating macrophages express high levels of TLRs and are the primary mediators of TLR-dependent inflammatory responses [70]. Tight regulation of TLR signalling is essential to avoid unchecked amplification of inflammation [71,72].

There is fairly consistent evidence for a role for TLRs in NAFLD/NASH. Mutation of TLR4 in mice leads to a decreased steatosis and resistance to NASH [73–76]. In agreement with this, TLR9 full-body knockout improves steatosis, inflammation and fibrosis in choline-deficient L-amino acid-defined (CDAA) diet [77] and in HFD fed mice [78]. However, in TLR2-deficient mice, the phenotype of NAFLD/NASH depends on the disease-inducing diet. TLR2-deficient mice fed a MCD exhibit an equivalent or a more severe steatohepatitis [79,80], whereas mice fed a HFD or CDAA diet are resistant to development of steatohepatitis and IR [67,81,82]. The contrasting results between the diets may arise from changes in the gut microbiota. These changes could be mitigated by either diet change or the fact that these studies were conducted at different facilities. Interestingly, TLR5 knockout mice spontaneously develop steatosis and obesity on a standard diet [83]. TLR5 recognises flagellin and expression of TLR5 in the intestinal mucosa plays a critical role for the development of metabolic syndrome [84,85], suggesting that the lack of TLR5 in the intestine can influence the development of NAFLD. Knockout of TLR7 in different murine fibrosis models increases fibrosis [54], suggesting that TLR7 has a protective role in disease progression. Overall, studies in mice demonstrate that PRRs are able to influence the progression of NAFLD at different stages and their involvement is PAMP/pathway dependent.

Inflammasomes govern caspase 1 activation and subsequent secretion of pro-inflammatory interleukins (IL-) 1β and IL-18 [86], which are critical in the development of liver damage in NAFLD [87]. TLR activation can provide one of the two signals (signal 1) required for inflammasome activation. Signal 2 is provided by stimuli from damaged cells. Elevated LPS in NAFLD [65,66] provides the critical signal 1 for sustained inflammasome activation [86], while toxic cell metabolites are a source of signal 2, as shown in macrophages [88]. Four types of inflammasomes, the NOD-like receptor family pyrin domain containing (NLRP) 1 and 3, CARD domain containing 4 (NLRC4) and absent in melanoma 2 (AIM2), have been described. A recent study demonstrated that NLRP3 inflammasome blockade using the selective small molecule NLRP3 inhibitor, MCC950, attenuates hepatic inflammation and fibrosis in murine mouse models [89].

9. Translational implications and future challenges

From what we have discussed, it is clear that IFN signalling can be a therapeutic target in NAFLD. The restricted expression pattern of type III IFN renders it a more attractive target than type I IFN [90]. Further investigation is needed to test whether the antagonism of type III IFN, or any of the components of its regulatory pathway, could be exploited as a therapeutic target to inhibit the progression of simple steatosis to NASH. Notably, we recently demonstrated a new role for zinc as a potent and specific inhibitor of IFN-λ3 signalling with strong inhibitory effects on hepatic inflammation [91]. Discovering specific modulators of type III IFN signalling and specific compounds, which can differentiate between anti-viral and pro-inflammatory effects, deserves further investigation. A recent study succeeded in developing a predictive score for liver fibrosis in NAFLD by including the *IFNL4* genotype in conjunction with clinical factors in multi-scoring diagnostic panels [92].

In spite of the strong evidence from human studies for a role of the type III IFNs in NAFLD progression, investigating the underlying functional mechanisms is hindered by the limitation of currently available murine models wherein the *IFNL4* gene is lost in contrast to most other mammals wherein the gene is preserved. In addition, mice do not express IFNλR1 in the liver and thus mice hepatocytes do not respond to

Table 1
The effect of genetic modification in NAFLD/NASH animal models on elements connected to interferon induction or signalling. HFD: High-fat-diet, MCD: methionine-/choline deficient diet, BDL: Bile duct ligation, CDAA: choline-deficient L-amino acid-defined diet, IR: insulin resistance.

	Animal model	Effect	Source
IFNαR1 −/−	HFD	Protected from steatosis and IR	[52]
IFNαR1 −/− *pDCs*	HFD	Protected from IR	[53]
IFNαR1 −/− *hepatocytes*	HFD, MCD	Increased steatosis, but no effect on metabolic parameters	[56]
IFNαR1 −/− *adipose tissue*	HFD, MCD	Increased IR, but no susceptibility to steatosis	[56]
IRF3 −/−	HFD	Protected from IR	[57]
IRF3 −/−	HFD	Increased IR and steatosis	[61]
IRF4 −/− *myeloid cells*	HFD	Increased IR	[62]
IRF5 −/− *myeloid cells*	MCD, BDL	Protected from hepatic fibrosis	[59]
IRF7 −/−	HFD	Increased insulin sensitivity	[58]
IRF9 −/−	HFD	Increased IR and steatosis	[63]
TLR4 mut	MCD, HFD Fructose	Decreased steatosis	[73,74,75,76]
TLR9 −/−	CDAA HFD	Decreased steatosis and fibrosis	[77,78]
TLR2 −/−	MCD HFD CDAA	Uncertain – diet dependent	[67,79,80,81,82]
TLR5 −/−	–	Spontaneous steatosis	[83]
TLR7 −/−	BDL, CCL_4-induced fibrosis	Increased liver fibrosis	[54]

type III IFN [33]. However, similar effects to IFN-α would be expected. As stated above, type I IFN involvement in NAFLD and NASH has been broadly studied in rodents. It is noteworthy that a porcine animal model might be convenient in further studies of type III IFNs since pigs possess *IFNL4* and according to RNAseq experiments the porcine liver express IFNλR1 [93]. Furthermore, human and porcine genetics have a high degree of similarity. A recent study in pigs using gene co-expression network analysis showed that liver network modules relevant to fatty acid unsaturation index were enriched in the type I IFN signalling pathway, emphasising the connections between both systems [94]. Even though, the domesticated pig is resistant to HFD induced NAFLD, the Ossabaw miniature swine develops metabolic syndrome and progressive histologic features of NASH when fed a diet high in fructose, saturated fat and cholesterol [95] and may in the future be a better animal model for studying NAFLD and the role of IFNs in this complex phenotype.

Acknowledgement

RH was supported by the Danish Council for Independent Research, Medical Research (grant 7016-00331B), the Novo Nordisk Foundation (Grant NNF15OC0017902) and the Riisfort foundation. ETD is funded by Danish Council for Independent Research (DFF-5053-00286). ME and JG are supported by the Robert W. Storr Bequest to the Sydney Medical Foundation, University of Sydney; a National Health and Medical Research Council of Australia (NHMRC) Program Grant (APP1053206) and Project grants (APP1107178 and APP1108422). KLT is supported by the Danish Council for Independent Research, Medical Research (DFF-6110-00536) and the Novo Nordisk Foundation (NNF15OC0017314). HG is supported by the NOVO Nordisk Foundation and "Savværksejer Jeppe Juhl og hustru Ovita Juhls mindelegat".

References

[1] Z. Younossi, et al., Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention, Nat. Rev. Gastroenterol. Hepatol. 15 (1) (2018) 11–20.
[2] L.A. Adams, K.D. Lindor, Nonalcoholic fatty liver disease, Ann. Epidemiol. 17 (11) (2007) 863–869.
[3] R.J. Wong, et al., Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States, Gastroenterology 148 (3) (2015) 547–555.
[4] A.J. McCullough, The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease, Clin. Liver Dis. 8 (3) (2004) 521–533 viii.
[5] B.Q. Starley, C.J. Calcagno, S.A. Harrison, Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection, Hepatology 51 (5) (2010) 1820–1832.
[6] D.L. White, F. Kanwal, H.B. El-Serag, Non-alcoholic fatty liver disease and hepatocellular cancer: a systematic review, Clin. Gastroenterol. Hepatol. 10 (12) (2012) 1342 1359.e2.
[7] C.P. Day, O.F. James, Steatohepatitis: a tale of two "hits"? Gastroenterology 114 (4) (1998) 842–845.
[8] H. Tilg, A.R. Moschen, Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis, Hepatology 52 (5) (2010) 1836–1846.
[9] M. Eslam, L. Valenti, S. Romeo, Genetics and epigenetics of NAFLD and NASH: clinical impact, J. Hepatol. (2017).
[10] J.F. Silverman, et al., Liver pathology in morbidly obese patients with and without diabetes, Am. J. Gastroenterol. 85 (10) (1990) 1349–1355.
[11] T. Andersen, P. Christoffersen, C. Gluud, The liver in consecutive patients with morbid obesity: a clinical, morphological, and biochemical study, Int. J. Obes. 8 (2) (1984) 107–115.
[12] A. Poonawala, S.P. Nair, P.J. Thuluvath, Prevalence of obesity and diabetes in patients with cryptogenic cirrhosis: a case-control study, Hepatology 32 (4 Pt 1) (2000) 689–692.
[13] J.M. Clark, A.M. Diehl, Hepatic steatosis and type 2 diabetes mellitus, Curr. Diab. Rep. 2 (3) (2002) 210–215.
[14] P. Gupte, et al., Non-alcoholic steatohepatitis in type 2 diabetes mellitus, J. Gastroenterol. Hepatol. 19 (8) (2004) 854–858.
[15] N. Assy, et al., Fatty infiltration of liver in hyperlipidemic patients, Dig. Dis. Sci. 45 (10) (2000) 1929–1934.
[16] D. Berkowitz, Metabolic changes associated with obesity before and after weight reduction, JAMA 187 (6) (1964) 399–403.
[17] G. Donati, et al., Increased prevalence of fatty liver in arterial hypertensive patients with normal liver enzymes: role of insulin resistance, Gut 53 (7) (2004) 1020–1023.
[18] E. Lau, D. Carvalho, P. Freitas, Gut microbiota: association with NAFLD and metabolic disturbances, Biomed. Res. Int. 2015 (2015) 979515.
[19] S. Romeo, et al., Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease, Nat. Genet. 40 (12) (2008) 1461–1465.
[20] L. Valenti, et al., Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease, Hepatology 51 (4) (2010) 1209–1217.
[21] J. Kozlitina, et al., Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease, Nat. Genet. 46 (4) (2014) p. 352-+.
[22] M. Eslam, et al., Diverse impacts of the rs58542926 E167K variant in TM6SF2 on viral and metabolic liver disease phenotypes, Hepatology 64 (1) (2016) 34–46.
[23] E.K. Speliotes, et al., Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits, Plos Genet. 7 (3) (2011).
[24] N.S. Abul-Husn, et al., A protein-truncating HSD17B13 variant and protection from chronic liver disease, N Engl. J. Med. 378 (12) (2018) 1096–1106.
[25] S. Buch, et al., A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis, Nat. Genet. 47 (12) (2015) p. 1443-+.
[26] R.M. Mancina, et al., The MBOAT7-TMC4 Variant rs641738 increases risk of nonalcoholic fatty liver disease in individuals of European descent, Gastroenterology 150 (5) (2016) 1219–1230 e6.
[27] K. Thabet, et al., MBOAT7 rs641738 increases risk of liver inflammation and transition to fibrosis in chronic hepatitis C, Nat. Commun. 7 (2016) 12757.
[28] K. Thabet, et al., The membrane-bound O-acyltransferase domain-containing 7 variant rs641738 increases inflammation and fibrosis in chronic hepatitis B, Hepatology 65 (6) (2017) 1840–1850.
[29] S. Petta, et al., IL28B and PNPLA3 polymorphisms affect histological liver damage in patients with non-alcoholic fatty liver disease, J. Hepatol. 56 (6) (2012) 1356–1362.
[30] S. Petta, et al., Interferon lambda 4 rs368234815 TT > deltaG variant is associated with liver damage in patients with nonalcoholic fatty liver disease, Hepatology 66 (6) (2017) 1885–1893.
[31] M. Eslam, et al., Interferon-lambda rs12979860 genotype and liver fibrosis in viral and non-viral chronic liver disease, Nat. Commun. 6 (2015) 6422.
[32] G. Schreiber, The molecular basis for differential type I interferon signaling, J. Biol.

Chem. 292 (18) (2017) 7285–7294.
[33] A. Wack, E. Terczynska-Dyla, R. Hartmann, Guarding the frontiers: the biology of type III interferons, Nat. Immunol. 16 (8) (2015) 802–809.
[34] H. Dickensheets, et al., Interferon-lambda (IFN-lambda) induces signal transduction and gene expression in human hepatocytes, but not in lymphocytes or monocytes, J. Leukoc. Biol. 93 (3) (2013) 377–385.
[35] C. Lauber, et al., Transcriptome analysis reveals a classical interferon signature induced by IFN lambda 4 in human primary cells, Genes Immun. 16 (6) (2015) 414–421.
[36] A.J. Muir, et al., A randomized Phase 2b Study of peginterferon lambda-1a for the treatment of chronic HCV infection, J. Hepatol. (2014).
[37] P. Hermant, T. Michiels, Interferon-lambda in the context of viral infections: production, response and therapeutic implications, J. Innate Immun. 6 (5) (2014) 563–574.
[38] R.K. Durbin, S.V. Kotenko, J.E. Durbin, Interferon induction and function at the mucosal surface, Immunol. Rev. 255 (1) (2013) 25–39.
[39] S.N. Chen, et al., Evolution of IFN-lambda in tetrapod vertebrates and its functional characterization in green anole lizard (Anolis carolinensis), Dev. Comp. Immunol. 61 (2016) 208–224.
[40] F.M. Key, et al., Selection on a variant associated with improved viral clearance drives local, adaptive pseudogenization of interferon lambda 4 (IFNL4), PLoS Genet. 10 (10) (2014) e1004681.
[41] L. Prokunina-Olsson, et al., A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus, Nat. Genet. 45 (2) (2013) 164–171.
[42] A.P. McFarland, et al., The favorable IFNL3 genotype escapes mRNA decay mediated by AU-rich elements and hepatitis C virus-induced microRNAs, Nat. Immunol. 15 (1) (2014) 72–79.
[43] Y. Tanaka, et al., Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C, Nat. Genet. 41 (10) (2009) 1105–1109.
[44] V. Suppiah, et al., IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy, Nat. Genet. 41 (10) (2009) 1100 U74.
[45] K.R. Smith, et al., Identification of improved IL28B SNPs and haplotypes for prediction of drug response in treatment of hepatitis C using massively parallel sequencing in a cross-sectional European cohort, Genome Med. 3 (8) (2011) 57.
[46] E. Terczynska-Dyla, et al., Reduced IFNlambda4 activity is associated with improved HCV clearance and reduced expression of interferon-stimulated genes, Nat. Commun. 5 (2014) 5699.
[47] P.Y. Bochud, et al., IL28B alleles associated with poor hepatitis C virus (HCV) clearance protect against inflammation and fibrosis in patients infected with non-1 HCV genotypes, Hepatology 55 (2) (2012) 384–394.
[48] M. Eslam, et al., IFN-lambda 3, not IFN-lambda 4, likely mediates IFNL3-IFNL4 haplotype-dependent hepatic inflammation and fibrosis, Nat. Genet. 49 (5) (2017) 795.
[49] M. Eslam, et al., IFN-lambda3, not IFN-lambda4, likely mediates IFNL3-IFNL4 haplotype-dependent hepatic inflammation and fibrosis, Nat. Genet. 49 (5) (2017) 795–800.
[50] M.H. Heim, R. Thimme, Innate and adaptive immune responses in HCV infections, J. Hepatol. 61 (1 Suppl) (2014) S14–S25.
[51] S. Shrivastava, et al., Elevated hepatic lipid and interferon stimulated gene expression in HCV GT3 patients relative to non-alcoholic steatohepatitis, Hepatol. Int. 10 (6) (2016) 937–946.
[52] M. Ghazarian, et al., Type I interferon responses drive intrahepatic T cells to promote metabolic syndrome, Sci. Immunol. 2 (10) (2017).
[53] T.D. Hannibal, et al., Deficiency in plasmacytoid dendritic cells and type I interferon signalling prevents diet-induced obesity and insulin resistance in mice, Diabetologia 60 (10) (2017) 2033–2041.
[54] Y.S. Roh, et al., Toll-like receptor 7-mediated type I interferon signaling prevents cholestasis- and hepatotoxin-induced liver fibrosis, Hepatology 60 (1) (2014) 237–249.
[55] K. Mitsumoto, et al., Time-course microarrays reveal early activation of the immune transcriptome in a choline-deficient mouse model of liver injury, Life Sci. 184 (2017) 103–111.
[56] V. Wieser, et al., Adipose type I interferon signalling protects against metabolic dysfunction, Gut (2016).
[57] M. Kumari, et al., IRF3 promotes adipose inflammation and insulin resistance and represses browning, J. Clin. Invest. 126 (8) (2016) 2839–2854.
[58] X.A. Wang, et al., Interferon regulatory factor 7 deficiency prevents diet-induced obesity and insulin resistance, Am. J. Physiol. Endocrinol. Metab. 305 (4) (2013) E485 95.
[59] F. Alzaid, et al., IRF5 governs liver macrophage activation that promotes hepatic fibrosis in mice and humans, JciInsight 1 (20) (2016).
[60] J.T. Qiao, et al., Activation of the STING-IRF3 pathway promotes hepatocyte inflammation, apoptosis and induces metabolic disorders in nonalcoholic fatty liver disease, Metabolism (2017).
[61] X.A. Wang, et al., Interferon regulatory factor 3 constrains IKKbeta/NF-kappaB signaling to alleviate hepatic steatosis and insulin resistance, Hepatology 59 (3) (2014) 870–885.
[62] J. Eguchi, et al., Interferon regulatory factor 4 regulates obesity-induced inflammation through regulation of adipose tissue macrophage polarization, Diabetes 62 (10) (2013) 3394–3403.
[63] X.A. Wang, et al., Interferon regulatory factor 9 protects against hepatic insulin resistance and steatosis in male mice, Hepatology 58 (2) (2013) 603–616.
[64] T. Csak, et al., Mitochondrial antiviral signaling protein defect links impaired antiviral response and liver injury in steatohepatitis in mice, Hepatology 53 (6) (2011) 1917–1931.
[65] Y. Ilan, Leaky gut and the liver: a role for bacterial translocation in nonalcoholic steatohepatitis, World J. Gastroenterol. 18 (21) (2012) 2609–2618.
[66] N. Enomoto, et al., Kupffer cell sensitization by alcohol involves increased permeability to gut-derived endotoxin, Alcohol Clin. Exp. Res. 25 (6 Suppl) (2001) 51S 4S.
[67] K. Miura, et al., Toll-like receptor 2 and palmitic acid cooperatively contribute to the development of nonalcoholic steatohepatitis through inflammasome activation in mice, Hepatology 57 (2) (2013) 577–589.
[68] T. Kawai, S. Akira, TLR signaling, Semin. Immunol. 19 (1) (2007) 24–32.
[69] I. Jialal, H. Kaur, S. Devaraj, Toll-like receptor status in obesity and metabolic syndrome: a translational perspective, J. Clin. Endocrinol. Metab. 99 (1) (2014) 39–48.
[70] D.W. Ye, et al., Toll-like receptor-4 mediates obesity-induced non-alcoholic steatohepatitis through activation of X-box binding protein-1 in mice, Gut 61 (7) (2012) 1058–1067.
[71] L.A.J. O'Neill, When signaling pathways collide: positive and negative regulation of Toll-like receptor signal transduction, Immunity 29 (1) (2008) 12–20.
[72] M. Fujihara, et al., Molecular mechanisms of macrophage activation and deactivation by lipopolysaccharide: roles of the receptor complex, Pharmacol. Ther. 100 (2) (2003) 171–194.
[73] C.A. Rivera, et al., Toll-like receptor-4 signaling and Kupffer cells play pivotal roles in the pathogenesis of non-alcoholic steatohepatitis, J. Hepatol. 47 (4) (2007) 571–579.
[74] A. Spruss, et al., Toll-like receptor 4 is involved in the development of fructose-induced hepatic steatosis in mice, Hepatology 50 (4) (2009) 1094–1104.
[75] T. Csak, et al., Deficiency in myeloid differentiation factor-2 and toll-like receptor 4 expression attenuates nonalcoholic steatohepatitis and fibrosis in mice, Am. J. Physiol. Gastrointest. Liver Physiol. 300 (3) (2011) G433 41.
[76] M. Poggi, et al., C3H/HeJ mice carrying a toll-like receptor 4 mutation are protected against the development of insulin resistance in white adipose tissue in response to a high-fat diet, Diabetologia 50 (6) (2007) 1267–1276.
[77] K. Miura, et al., Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1beta in mice, Gastroenterology 139 (1) (2010) 323 34.e7.
[78] I. Garcia-Martinez, et al., Hepatocyte mitochondrial DNA drives nonalcoholic steatohepatitis by activation of TLR9, J. Clin. Invest. 126 (3) (2016) 859–864.
[79] G. Szabo, et al., Modulation of non-alcoholic steatohepatitis by pattern recognition receptors in mice: the role of toll-like receptors 2 and 4, Alcohol Clin. Exp. Res. 29 (11 Suppl) (2005) 140s 145s.
[80] C.A. Rivera, et al., Toll-like receptor-2 deficiency enhances non-alcoholic steatohepatitis, BMC Gastroenterol. 10 (2010) 52.
[81] J.A. Ehses, et al., Toll-like receptor 2-deficient mice are protected from insulin resistance and beta cell dysfunction induced by a high-fat diet, Diabetologia 53 (8) (2010) 1795–1806.
[82] R.W. Himes, C.W. Smith, Tlr2 is critical for diet-induced metabolic syndrome in a murine model, Faseb J. 24 (3) (2010) 731–739.
[83] M. Vijay-Kumar, et al., Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5, Science 328 (5975) (2010) 228–231.
[84] M. Vijay-Kumar, et al., Deletion of TLR5 results in spontaneous colitis in mice, J. Clin. Invest. 117 (12) (2007) 3909–3921.
[85] S.E. Letran, et al., TLR5-deficient mice lack basal inflammatory and metabolic defects but exhibit impaired CD4 T cell responses to a flagellated pathogen, J. Immunol. (Baltimore, Md. : 1950) 186 (9) (2011) 5406–5412.
[86] H. Guo, J.B. Callaway, J.P. Ting, Inflammasomes: mechanism of action, role in disease, and therapeutics, Nat. Med. 21 (7) (2015) 677–687.
[87] G. Szabo, A. Iracheta-Vellve, Inflammasome activation in the liver: focus on alcoholic and non-alcoholic steatohepatitis, Clin. Res. Hepatol. Gastroenterol. 39 (Suppl 1) (2015) S18–S23.
[88] H. Wen, et al., Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling, Nat. Immunol. 12 (5) (2011) 408–415.
[89] A.R. Mridha, et al., NLRP3 inflammasome blockade reduces liver inflammation and fibrosis in experimental NASH in mice, J. Hepatol. 66 (5) (2017) 1037–1046.
[90] M. Eslam, J. George, Targeting IFN-: therapeutic implications, Expert Opin. Ther. Targets 20 (12) (2016) 1425–1432.
[91] S.A. Read, et al., Zinc is a potent and specific inhibitor of IFN-lambda 3 signalling, Nat. Commun. (2017) 8.
[92] M. Eslam, et al., FibroGENE: a gene-based model for staging liver fibrosis, J. Hepatol. 64 (2) (2016) 390–398.
[93] M. Li, et al., Comprehensive variation discovery and recovery of missing sequence in the pig genome using multiple de novo assemblies, Genome Res. 27 (5) (2017) 865–874.
[94] J. Zhang, et al., Transcriptome analyses reveal genes and pathways associated with fatty acid composition traits in pigs, Anim. Genet. 48 (6) (2017) 645–652.
[95] T. Liang, et al., Liver injury and fibrosis induced by dietary challenge in the Ossabaw miniature Swine, PLoS One 10 (5) (2015) e0124173.