Non-Alcoholic Fatty Liver Disease – A Brief Insight into Pathogenesis and Review of Recent Reports on Therapeutic Targets

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Abstract
Non-alcoholic fatty liver disease (NAFLD) is a complication of global prevalence occurring due to defective regulation of hepatic lipid metabolism. Currently, NAFLD is being viewed as an emerging epidemic by virtue of increase in obesity cases. Accumulation of excess fatty acid in the liver, leads to activation of an array of inflammatory signals, resulting in hepatic steatosis. Inadequately treated steatosis would progress to non-alcoholic steatohepatitis (NASH) marked by severe hepatic inflammation and fibrosis. In the light of rising NAFLD cases and lack of a validated therapy, formulation of novel and efficient treatment strategies are in high demand. Several factors including inflammatory cytokines, chemokines and receptors are involved in execution of the inflammatory process in NASH progression. A clarified comprehension related to the mode of action and interplay between these factors is indispensible for an efficient therapeutic approach for NAFLD. This review is focused on the key factors involved in NAFLD progression and their potential as therapeutic targets.

ABBREVIATIONS
NAFLD: Non-Alcoholic Fatty Liver Disease; NASH: Non-Alcoholic Steatohepatitis; VLDL: Very Low Density Lipoproteins; TNF-α: Tumor Necrosis Factor-α; JNK1: C-Jun N-Terminal Kinase 1; TGF-β: Transforming Growth Factor-β; IκB: Inhibitory Factor Kappa B; IL: Interleukin; CRP: C-Reactive Protein; CT: Computerized Tomography; FXR: Farnesoid X Receptor; CCL/CXCL: Chemokine Ligand; CCR/CXCR: Chemokine Receptor; PPAR: Peroxisome Proliferator Activated Receptor; KLF: Kruppel Like Factor 6; HDL: High Density Lipoprotein

INTRODUCTION
Globally there has been an upsurge in health complications related to hepatic disorders by virtue of high fat diet consumption of the general population and predisposing genetic factors. Non-alcoholic fatty liver disease is one of the leading causes of chronic liver diseases affecting one third of the population in developed countries [1]. Non-alcoholic fatty liver disease (NAFLD) is a term blaneting wide spectrum of observed clinical hepatic complications ranging from mild steatosis to fibrotic and cirrhotic liver [1,2]. It is characterized by accumulation of excess fat (>5%) in the hepatocytes in the form of triglycerides, leading to detectable enlargement of liver and altered metabolic symptoms [3].

NAFLD is primarily associated with impaired lipid metabolism attributed to genetic and physiological causes, the exact origin of which is yet to be comprehended completely [4,5]. The ‘two hit’ theory is considered as the most acceptable version of NAFLD pathogenesis. The first hit is due to an imbalance between uptake of free fatty acids, de novo – lipogenesis and their usage through β-oxidation and secretion into circulation by very low density
lipoproteins (VLDL) which results in excess lipid accumulation in liver. The second hit is the consequence of excess hepatic lipid accumulation which leads to oxidative stress which further triggers the release of inflammatory cytokines, adipokines and mitochondrial dysfunction leading to steatohepatitis [6,7]. The changes in hepatocytes are observed as a spectrum ranging from simple lipid accumulation in vacuoles to aggressive fibrotic lesions [8].

The transition from NAFLD to non-alcoholic steatohepatitis (NASH) is the major hallmark in the progressive pathogenesis [8, 9]. The inflammatory signals in liver lead to activation of hepatic stellate cells, which leads to secretion of extracellular matrix resulting in fibrotic lesions [10]. Studies show that approximately 29% of patients suffering from NASH end up with fibrosis in 10 years [11]. The physiological steatosis stage resulting due to lipid deposition in hepatocytes is highly reversible. However, the continued stress to hepatocytes due to diminished efflux of triglycerides (TGs) triggers the oxidative stress [12]. Lipid peroxidation of liver cells is considered as a significant contributor to initiation of inflammatory signals [13].

Pathophysiological factors such as insulin resistance, obesity and genetic predisposition aid in progression of NASH [14]. Several studies have reported the role of inflammatory mediators viz., cytokines, chemokines, adipokines and transcriptional factors [15, 16]. However, an overall understanding of these signaling cascades and molecular players is essential for better interpretation and designing of future therapy for NAFLD. This review would focus on some of the key players in the inflammatory signaling and therapeutic targets involved in NAFLD.

MOLECULAR TARGETS

Cytokines

Cytokines are messengers secreted by various cell types in the body in response to a wide variety of physiological stimulus [17]. Cytokines aid in normal physiological processes such as growth, differentiation, hematopoesis, as well as several inflammatory and immune responses [18]. Their involvement in execution of inflammatory reactions in several pathologies such as cardiovascular disease, rheumatoid arthritis, NAFLD, etc., have been reported earlier [19, 20]. The cytokines as present in minimal levels in hepatic circulation during normal physiological status and are necessary for hepatic homeostasis [17]. However, the cytokines have been observed to play an active role in mediating the inflammatory progression of NAFLD as characterized by apoptotic and necrotic lesion in liver leading to fibrosis [15]. The cytokines involved in hepatic inflammation, are categorized under several subfamilies – Tumor necrosis factor-α (TNF-α), Tumor growth factor β (TGF-β), Interleukins and chemokines.

Tumor Necrosis Factor α

TNF-α is a pro-inflammatory cytokine secreted by several cell types such as neutrophils, macrophages, T and B lymphocytes, endothelial cells, mast cells, fibroblasts etc., In liver, hepatocytes and Kupffer cells are the major contributors of TNF- α [21]. TNF-α plays a central role in the initiation of inflammatory cascade and its progression from steatosis to steatohepatitis [22]. Experiments with mice models for obesity have shown the importance of TNF-α in NAFLD, where anti-TNF-α –drug therapy showed promising results [23, 24]. Increased free fatty acid level in obesity stimulates the hepatocytes to secrete TNF-α, which elicits the free fatty acid induced expression of inflammatory genes [23]. A positive correlation has been observed between TNF-α level in serum and degree of fibrosis in patients with NAFLD [25].A study with pentoxyfiline - an inhibitor of TNF-α has shown suppressive effect on elevation of serum transaminases and triglycerides in experimental NAFLD induced rats. The study also demonstrated that NAFLD induced TNF-α expression stimulates endoplasmic reticulum stress, which further mediate the progression of steatosis to fibrosis [26].

c-Jun N-terminal Kinase 1 (JNK1) a stress activated protein kinase is activated by TNF-α which leads to initiation of an autocrine/paracrine loop resulting in enhanced TNF-α production in liver. TNF-α activates inhibitory kappa b kinase β (IKKβ) which phosphorylates IκB resulting in the translocation of NF-κB into nucleus. TNF-α - NF-κB axis mediated proinflammatory cytokine activation has been implemented in the pathogenesis of NAFLD [27]. Study with NF-κB and mitogen activated protein kinase (MAPKs) inhibition has shown to suppress TNF-α secretion, supporting the establishment of TNF-α - NF-κB loop for inflammatory mediation [28].

Transforming growth factor β

TGF-β is a cytokine secreted by hepatocytes and Kupffer cells in response to degradation changes in liver. TGF-β activates the resting stellate cells by transforming them into active myofibroblasts, which secrete extracellular matrix protein to initiate the fibrosis process [29]. Earlier studies have shown up regulated expression of TGF-β following experimentally induced hepatic damage such as in CCl4 poisoning [30, 31]. Elevated TGF-β mRNA expression levels have been found in patients with liver fibrosis. Earlier studies have shown that TGF-β is an early marker for the progression of steatohepatitis [32]. Hence, detection of TGF-β level would be helpful in marking NASH stage of NAFLD. Studies have observed polymorphisms in TGF-β1 gene in obese NAFLD patients with advanced stages of hepatic fibrosis [33]. Xiao and Ho have recently reported that administration of Epigallocatechin gallate (EGCG) reduced hepatic severity in NAFLD by suppression of TGF/SMAD pathway [34]. Studies with TGF-β receptor II deficient mice showed protective effect against experimentally induced NAFLD with methionine and choline deficient (MCD) diet, which was mediated through smad2 activation [35].

Interleukins

Interleukin-6 is a proinflammatory cytokine which has been implicated in metabolic syndrome [36]. IL-6 also plays several other functions such as inducer of immune response, hematopoesis and oncogenesis [37]. Certain studies have reported IL-6 to be hepatoprotective and mediate hepatic regeneration after partial hepatectomy in mice [38, 39]. IL-6 has also been considered to reduce hepatic oxidative stress and to curtail mitochondrial dysfunction. However, studies have reported the role of IL-6 as an acute phase inflammatory mediator leading to secretion of several inflammatory serum proteins [40]. Hence, the possibility of its role in pathogenesis of NAFLD cannot
A positive correlation has been found in patients with NASH and circulating IL-6 level [41]. Studies with IL-6 knockout mice models have shown reduced severity when subjected to experimental NAFLD [42]. Yamaguchi et al, has shown that inhibition of IL-6 receptor with Tocilizumab, enhanced hepatic steatosis but protected against extensive hepatic damage in MCD diet induced NASH [43]. Recently, Hamirani et al, have observed a positive correlation between C – reactive protein (CRP) and IL-6 levels and increased liver fat accumulation in NAFLD patients as verified with CT-scans [44].

Interleukin-10 is considered as an anti-inflammatory cytokine with regulatory role over inflammation and pathophysiology of liver and other organs [19]. IL-10 is produced by hepatocytes, Kupffer cells and stellate cells in liver. But the exact role of IL-10 is yet to be defined. IL-10 has been shown to inhibit cell mediated inflammatory changes in liver [19]. Mc Mahan et al, found that INT-767 an agonist of farnesoid X receptor (FXR) increases IL-10 expression, which promotes the alternative activation of macrophage population. These macrophage population activated by IL-10 are found to be anti-inflammatory and hepatoprotective [45].

Chemokines

Chemokines are cytokines which act as chemo-attractants for leukocyte trafficking in the inflammatory sites [46]. Chemokines are classified into four subfamilies (C, CC, CXC and CX3C) according to the arrangement of the N-terminal conserved cysteine residues. They are secreted by variety of cell types such as hepatocytes, stellate cells, endothelial cells and smooth muscle cells [47]. Chemokines execute their action by binding to the G-protein coupled receptors on the target cells. Studies have shown up regulated expression of chemokines and chemokines receptors in liver of patients with advanced stages of NAFLD portraying severe steatosis [48].

CCL2 is a crucial chemokine secreted by macrophages, endothelial cells and hepatic stellate cells in response to inflammatory stimulus. The elevation in free fatty acid level stimulates NF-κB activation induced secretion of inflammatory cytokines including TNF-α and CCL2 [49, 50]. It executes the target cell activation by binding to its receptor CCR2 [51]. Inhibition of CCL2/CCR2 pathway in mice has shown improvement of hepatic steatosis and adipocytes inflammation [52, 53]. Obstfeld et al, elucidated the role of CCL2 in progression of hepatic steatosis. They found that CCL2 expression in obesity activates and recruits a population of myeloid cells which leads to steatohepatitis [54]. In a recent study, Cynis et al have observed that the inhibition of glutaminyl cyclases - the enzymes necessary for maturation of cytokines to active form, improved the hepatic steatosis condition in experimental NAFLD [55].

CCL5 is associated with chronic inflammatory conditions and its relation with NAFLD has recently been described [56]. CCL5 are mainly secreted by hepatocytes and their release is mediated by excess lipid deposition in liver [57].
essential for progression of hepatic steatosis to fibrosis and the studies blocking CCR5 have shown to be efficient in reducing the severity of NASH [58]. Certain studies have reported association of increases CXCL8 levels in serum with NASH [59, 60]. Similarly, CXCL9 and CXCL10 interact with a common receptor CXCR3 [61]. In NASH their levels are higher in liver and aid the migration of inflammatory cell types into hepatic parenchyma, thus promoting hepatic fibrosis [62, 63]. Inhibition of CXCL9 – CXCR3 has proven to be an efficient therapeutic target [64].

Peroxisome proliferator activated receptors (PPARs)

PPARs belong to nucleic acid receptor superfamily which consists of PPARα, PPARβ/δ and PPARγ - expressed in mammals [65]. PPARα is synthesized extensively in metabolically active sites such as liver, intestine, muscle and brown adipose tissue. It mainly controls the transport of fatty acids and their β-oxidation, thereby depleting the hepatic lipid storage [66, 67]. PPARα activation inhibits NF-κB translocation mediated inflammatory cascade and release of C-reactive protein [68]. Previous experiments with PPARα agonists in MCD diet fed mice have proven beneficial in prevention of steatosis progression [69, 70]. PPARα agonism has also been reported to revert fibrosis by suppression of inflammatory signals and reduced expression of fibrotic marker. PPARα knockout mice have demonstrated increased susceptibility to NASH [71]. Bechmann et al, have reported the role of Kruppel like factor 6 (KLF6) a transcription factor which elicits pathology of NAFLD through PPARα post-transcriptional activation. KLF6 deficient mice were protected from high fat diet induced hepatic damage compared to normal mice [72]. PPARα activation has a direct anti-inflammatory role irrespective of hepatic triglyceride levels [73].

PPARγ is expressed in adipocytes where it regulates fatty acids uptake and increases insulin sensitivity [74]. Increased expression of PPARγ has been found in steatotic liver, as elucidated by previous studies due to its activation of de novo lipogenesis [75]. Studies with PPARγ deletion in mice have shown to protect liver against steatosis, suggesting that PPARγ has a prosteatotic role [76]. Contrastingly, other studies have elucidated a protective effect of PPARγ against NAFLD [77]. Adenovirus mediated overexpression of PPARγ has shown to protect liver against steatosis and fibrosis [78]. PPARγ expression has shown to possess anti-fibrotic activity by suppression of stellate cell proliferation [79]. Though the role of PPARβ/δ in NAFLD remains unclear, it has been implicated as a metabolic regulator with roles in fatty acid transport and glucose homeostasis [80]. Studies have exemplified its role in attenuation of macrophage inflammatory response and elevation of plasma HDL concentrations [81]. Treatment with PPARβ/δ agonist GW501516 improves the hepatic condition by suppression of steatosis and inflammatory signals by regulation of lipid metabolism in MCD diet fed mice [82]. CCL5, toxicity in liver triggers PPARβ/δ expression, attributing to the inductive hepatic expression of PPARβ/δ as a protective response [83]. Recently, Staels et al, have reported beneficial role of PPARβ/δ agonist GT505 in western diet fed hApoE2 knock-in /PPAR-α knockout mice. GT505 demonstrated protective effect on liver irrespective of high fat diet feeding or exposure to CCL4 [69].

CONCLUSION

The progression of steatosis to steatohepatitis in NAFLD is an intricate process organized by complex signaling cascades of metabolic and inflammatory factors. Current review provides an overview of crucial factors involved in mediating the inflammatory progression and also an update on the inhibitor/agonist studies targeting specific factors to achieve suppression/inhibition of steatosis progression. Though several studies have reported the beneficial effects of inhibitors/agonists of specific factors on prevention or suppression of NAFLD, further understanding of the role of inflammatory signals and molecular crosstalk is essential to devise flawless therapeutic designs. Moreover, the adverse effects of such therapeutic agents shouldn’t be ignored to prevent complications arising post therapy. Future studies exploring multiple inflammatory targets are necessary for improved combinatorial therapies for better management of NAFLD.

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