4 Chemokines and Chemokine Receptors in the Development of NAFLD

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Abstract

Chemokines are chemo-attractants for leukocyte trafficking, growth, and activation in injured and inflammatory tissues. The chemokine system is comprised of 50 chemokine ligands and 20 cognate chemokine receptors. In the context of liver diseases, leukocytes, hepatocytes, hepatic stellate cells, endothelial cells, and vascular smooth muscle cells are capable of producing chemokines. Chemokine receptors are typically expressed in various leukocyte subsets. Given that inflammation is a critical factor for the transition from simple steatosis to non-alcoholic steatohepatitis (NASH), and fibrosis, the chemokine system may play a prominent role in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). Indeed, accumulating evidence shows elevated expression of chemokines and their receptors in the livers of obese patients with advanced steatosis and NASH. This chapter will discuss the underlying molecular mechanisms and the therapeutic potential of the chemokine systems in the pathogenesis of NAFLD. Among chemokines, we will highlight CCL2, CCL5, CXCL8-10, CX3CL1, and CXCL16 as pivotal mediators in the development of steatosis, NASH, and fibrosis.

Keywords

Chemokines · Chemokine receptors · NAFLD · NASH · Fibrosis

4.1 Chemokines and NAFLD

Chemokines are chemotactic cytokines which are small heparin-binding proteins. They act as chemo-attractants for leukocyte trafficking, growth, and activation in inflammatory sites [1, 2]. Approximately 50 chemokines were identified and classified into four subfamilies (C, CC, CXC and CX3C) based on the arrangement of the N-terminal conserved cysteine residues. Twenty cognate chemokine receptors have been identified as relevant in the context of liver diseases (Table 4.1) [3]. Various cell types, including leukocytes, hepatocytes, hepatic stellate cells, liver sinusoidal endothelial cells, and vascular smooth muscle cells, can produce chemokines [4]. Chemokine receptors are typically expressed in various leukocyte subsets and immune cells. Chemokine receptors are seven transmembrane

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Table 4.1 Important chemokine and chemokine receptor pathways in NAFLD

Chemokine	Alternative name	Cellular source of chemokine	Chemokine receptor	Target cell	Overall effect in NAFLD
CCL2	MCP-1	Hepatocytes, Kupffer cells, HSCs	CCR2	Monocytes/ macrophages, HSCs	Promition
CCL5	RANTES	HSCs, macrophages, hepatocytes, platelets	CCR1, CCR5	NK, Th1, CD8 T, HSCs	Promotion
CXCL8	IL-8	Hepatocytes, macrophages	CXCR1, CXCR2	Neutrophils, monocyte	Promotion
CXCL9	MIG	Hepatocytes, LSECs, HSCs/MFs	CXCR3	NK, Th1, Th17	Promotion
CXCL10	IP-10	Hepatocytes, LSECs, HSCs/MFs	CXCR3	NK, Th1, Th17, HSCs	Promotion
CX3CL1	Fracktalkine	Hepatocytes, HSCs/MFs, LSECs	CX3CR1	Monocytes/ macrophages	Controversial
CXCL16		LSECs, Kupffer cells	CXCR6	NKT cells	Promotion

HSC hepatic stellate cell, *LSEC* liver sinusoidal endothelial cell, *MF* myofibroblast

G-protein coupled receptors. Upon binding of chemokines to the corresponding chemokine receptors, the downstream intracellular cascades, such as phosphatidylinositol 3-kinase, small Rho guanosine triphosphatase, and cellular calcium influx pathways, are activated, which increases in the avidity of leukocyte integrins that promote leukocyte's interactions with adhesion molecules expressed on sinusoidal endothelial cells, such as intercellular adhesion molecules (ICAMs) and vascular cell adhesion molecules (VCAMs), thereby enabling leukocyte adhesion and subsequent extravasation [5]. Secreted chemokines require an interaction with glycosaminoglycans (GAGs) which are bound to the extracellular matrix and endothelial surface. This interaction locally immobilizes and retains chemokines, creating a concentration gradient that allows a coordinated migration of leukocytes toward inflammatory sites [6]. Infiltrated leukocytes produce inflammatory cytokines that further stimulate hepatic immune cells including liver resident macrophages and recruited circulating monocytes, hepatic stellate cells, and hepatocytes, which enhances liver inflammation (Fig. 4.1). Enhanced liver inflammation contributes to the enhancement of hepatocyte lipid accumulation, the transition from simple steatosis to non-alcoholic steatohepatitis (NASH), and the progression from steatohepatitis to fibrosis. Chemokine systems not only act as chemo-attractants, but also have potential to directly stimulate hepatocytes and hepatic stellate cells to enhance their biological activities, such as lipid accumulation and collagen production, respectively. An Accumulation of data has shown evidence of elevated expression of chemokines and their receptors in the livers of obese patients with advanced steatosis and NASH [7]. Inflammation plays a critical role in the progression of non-alcoholic fatty liver disease (NAFLD). Thus, the chemokine systems may play various prominent roles in the pathogenesis of NAFLD [8].

Obesity or western-style diets lead to insulin resistance, adipokine imbalance, and mobilization of lipotoxic free fatty acids to the liver. In the context of liver, fat accumulation contributes to activation of Kupffer cells, which together with hepatocytes and hepatic stellate cells (HSCs) amplify inflammation via production of various chemokines (CCL2, CCL5, CXCL1, CXCL2, CXCL8, CXCL9, and CXCL10), and recruitment of immune cells (monocytes, activated T cells, NKT cells, neutrophils) into the liver. Chemokines have also been directly implicated in the further accumulation of lipids within hepatocytes and collagen deposition by activated HSCs.

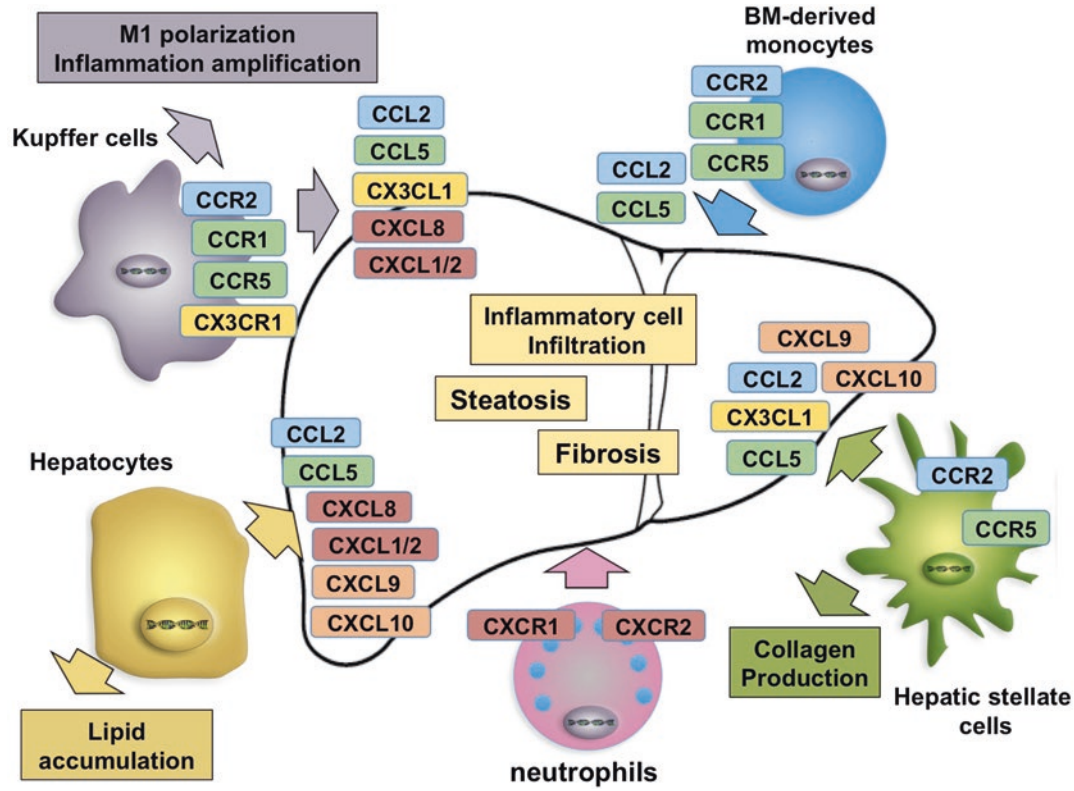

Fig. 4.1 Chemokines in the pathogenesis of nonalcoholic fatty liver disease

4.2 CCL2 (MCP-1) – CCR2

CCL2, also known as Monocyte Chemotactic Protein-1 (MCP-1), is a potent chemoattractant secreted by macrophages, endothelial cells, hepatic stellate cells, and vascular smooth muscle cells in response to inflammatory stimulus, such as interleukin (IL)-1β, tumor necrosis factor (TNF) α, and Toll-like receptor (TLR) ligands (e.g. lipopolysaccharide [LPS]) [9–11]. In pathological settings, hepatocyte CCL2 expression is associated with hepatocyte lipid accumulation in a diet-induced mouse NASH model [12]. In NAFLD, the increases of free fatty acids and activation of TLRs contributes to CCL2 production through NF-κB [13, 14]. In high-fat diet feeding conditions, hepatic CCL2 is upregulated and recruits a subset of myeloid cells, in turn promoting NASH development [15]. Deletion of CCL2 suppresses steatosis and insulin resistance in a diet-induced obese mouse model [16]. Since CCL2 activates the target cells through binding to its receptor CCR2 [17], CCR2 inactivation is expected to show a similar liver phenotype as with CCL2 inactivation. In mice, genetic deletion or pharmacological inhibition of CCR2 has shown the amelioration of inflammation and fibrosis in NASH and insulin resistance by inhibiting the recruitment of CCR2 expressing bone marrow-derived monocytes [14, 18, 19]. In early NAFLD, CCL2 produced from Kupffer cells (liver resident macrophages) is required for recruiting Ly6C positive circulating bone marrow-derived monocytes into the liver, which promotes liver inflammation [14]. Not only do macrophages/monocytes play a role on hepatic stellate cell recruitment and activation through inflammatory and fibrogenic cytokine production,

but CCL2 and CCR2 also directly play a role in hepatic stellate cell recruitment and activation that promotes liver fibrosis [20–22]. In NASH progression, adipose tissue macrophages also play an important role. In obesity, macrophages are infiltrated in adipose tissues, in which the CCL2-CCR2 system plays a major role [15]. Infiltration and activation of adipose tissue macrophages release inflammatory cytokines to the systemic circulation, which contributes to liver inflammation progression. In mice, the overexpression of CCL2 in adipose tissue promotes hepatic triglyceride levels and insulin resistance [16]. In human NASH livers, the upregulation of CCL2 was observed [23]. Another study demonstrated that CCL2 production is positively correlated with hepatic fat content in NAFLD patients [24]. These translational studies show additional evidence of the importance of the contribution of the CCL2/CCR2 pathway in the progression of NASH.

4.3 CCL5 (RANTES) – CCR1 and CCR5

CCL5 is also associated with chronic inflammatory diseases, such as NAFLD and liver fibrosis [25]. CCL5 is secreted by various cell types including macrophages, hepatocytes, hepatic stellate cells, and endothelial cells. Excessive lipid accumulation in the liver induces CCL5 production [26]. CCL5 is mainly involved in the migration of T-cells, monocytes, neutrophils, and dendritic cells through binding to its receptors CCR1, CCR3, and CCR5. The association of CCL5 with NAFLD has extensively been discussed in human and mouse studies. Hepatic expression of CCL5 is increased in a murine model of NASH and in obese patients [27, 28]. It should be noted that hepatocytes are a major source of CCL5 in NAFLD [26], suggesting that hepatic lipid accumulation mediates CCL5 release. CCL5 is required for the progression of liver fibrosis through binding to CCR1 and CCR5 [29, 30]. CCR1 is predominantly expressed in liver macrophages while CCR5 is expressed in both liver macrophages and hepatic stellate cells. However, these receptors have not been observed in hepatocytes. In liver fibrosis, CCR5 plays a dominant role in hepatic stellate cells, but not in liver macrophages, for their migration and activation by stimulating with CCL5 [29, 30]. CCR5 also plays a pivotal role in the recruitment of M1-type macrophages and their M1 polarization in adipose tissues, which contributes to insulin resistance and subsequently promotes the development of steatohepatitis [31]. These studies suggest that CCL5/CCR5-mediated signaling contributes to the development of hepatic steatosis, inflammation, fibrosis, and insulin resistance through monocyte/macrophage recruitment and stellate cell activation.

4.4 CXCL8 (IL-8), CXCL1, and CXCL2 – CXCR1 and CXCR2

CXCL8 is a CXC chemokine subfamily secreted by inflammatory cells and endothelial cells, and is also known as IL-8. IL-8 is currently identified only in humans, but not in mice. It is suggested that IL-8 homologues, CXCL1 and CXCL2, substitute the role of IL-8 in mice. These chemokines mainly regulate neutrophil recruitment to inflammatory sites. The serum levels of CXCL8 were significantly higher in NASH patients compared to simple steatosis patients or healthy controls [32]. Moreover, one study demonstrated that serum levels of CXCL8 were higher in subjects with NAFLD as compared to obese and non-obese patients [33]. Conversely, another study also demonstrated no association between serum CXCL8 and NAFLD [34]. More careful and intensive investigations on the function of CXCL8 in the pathogenesis of human NAFLD should be performed in the future. Since CXCL8 was cloned only in humans, the mechanistic analysis is limited. In contrast, the studies of its receptor, CXCR2, that can be activated by its alternative ligands CXCL1, CXCL2, and CXCL5 have been investigated in NAFLD mouse models. In the NAFLD mouse model and in NAFLD patients, circulating and hepatic levels of lipocalin-2 (LCN2), a glycoprotein, are increased.

LCN2 mediates liver injury and inflammation through neutrophil recruitment and CXCR2 in NASH mouse models [35]. We have recently demonstrated that hepatic CXCL1 levels are induced in a TLR4-MyD88-dependent manner and that increased CXCL1 is involved in hepatic neutrophil infiltration, which promotes NASH and liver fibrosis [36]. These studies demonstrate the importance of CXCR1/CXCR2-mediated neutrophil and macrophage recruitment in the development of NAFLD.

4.5 CXCL9/MIG and CXCL10/ IP-10 – CXCR3

CXCL9 and CXCL10 bind to CXCR3 as a common receptor, which is highly expressed in macrophages, activated T cells, memory T cells, and natural killer cells [37]. CXCL9 and CXCL10 promote the recruitment of these cell types. However, these chemokines are generally undetectable in most non-lymphoid tissues under physiological conditions. In pathologic conditions, hepatic endothelial cells produce high levels of CXCL9, leading to the migration of the CXCR3-expressing lymphocytes [38]. Moreover, the expression levels of CXCL9 were increased in the livers of NASH patients and mouse NASH models [39, 40]. CXCL10 is produced in macrophages, monocytes, hepatocytes, hepatic stellate cells, and endothelial cells [41]. CXCL10 plays a pivotal role in the pathogenesis of experimental steatohepatitis through induction of inflammation, oxidative stress, and lipogenesis [42]. A recent study reported that extracellular vesicles (EVs) released from lipid-accumulated hepatocytes contain CXCL10 that plays a central role in macrophage recruitment in NAFLD. The production of EVs containing CXCL10 is mediated by MLK3 in hepatocytes [43]. This study provided new evidence that EVs act as vehicles for delivering chemokines as cargos to target organs and cells. Consistently, CXCL10 has been proposed as a potential therapeutic target for the treatment of NASH, progressive liver injury, insulin resistance, and incident diabetes [41, 44].

4.6 CX3CL1/ Fractalkine – CX3CR1

CX3CL1, also known as Fractalkine, is a membrane-bound type of chemokine. CX3CL1 is involved in cell recruitment and cell survival through binding to CX3-chemokine receptor 1 (CX3CR1) [45]. In addition, CX3CR1-expressing monocytes circulate in the steady state and differentiate into alternatively activated macrophages [46]. In the liver, CX3CL1 is produced in Kupffer cells/macrophages and hepatic stellate cells [47]. The responsible receptor CX3CR1 is mainly expressed in Kupffer cells/macrophages. The CX3CL1-CX3CR1 interaction induces liver macrophage apoptosis and alternatively acquires anti-inflammatory properties of Kupffer cells/macrophages, which contribute to the negative regulation of liver inflammation and fibrosis [47, 48]. However, the role of CX3CL1/CX3CR1 signaling in NAFLD is still controversial. In an experimental mouse model, CX3CR1 has been reported to protect from excessive hepatic steatosis and inflammation, as well as systemic glucose intolerance through maintaining intestinal homeostasis [49]. Moreover, decreased CX3CL1/CX3CR1 pathway has been suggested to be a mechanism underlying β cell dysfunction in type 2 diabetes [50]. Conversely, CX3CR1+ moDCs (monocyte-derived inflammatory dendritic cells) have a pathologic role in the progression of NASH. The underlying mechanism that the study demonstrated is that the worsening of parenchymal injury was driven by an elevation in hepatic and circulating TNFα levels [51]. This discrepancy might be explained by the different roles of the CX3CL1/CXC3CR1 axis in different cell types.

4.7 CXCL16 – CXCR6

Previous studies demonstrated that the chemokine receptor, CXCR6, and its cognate ligand, CXCL16, control the patrolling of natural killer T (NKT) cells in liver sinusoids to maintain liver homeostasis [52]. In humans, higher CXCR6+ T

cells have been detected in the blood of patients with hepatitis C virus infection compared to healthy controls [53]. CXCL16 is expressed in hepatocytes and bile duct epithelial cells of patients with liver disease [53], as well as in murine liver sinusoidal endothelial cells [52]. CXCR6 promotes the infiltration of hepatic NKT cells and inflammatory macrophages, thereby promoting liver inflammation in experimental NAFLD [54, 55]. Indeed, CXCR6 gene expression was positively correlated with the inflammatory activity and ALT levels in patients with NAFLD [55] and injured hepatocytes had increased expression of CXCL16, a ligand of CXCR6, suggesting that the CXCL16-CXCR6 interaction plays a role in the pathogenesis of NAFLD [55].

4.8 Chemokines and Chemokine Receptors As Therapeutic Targets for the Treatment of NAFLD

It has been shown that pharmaceutical inhibition of CCR2 prevents the infiltration of the CCR2-expressing Ly6C-positive monocytes, resulting in an inhibition of NASH-mediated liver inflammation and fibrosis [14]. Consistently, pharmacological blockade of CCL2/CCR2 signaling in several mouse models of metabolic diseases significantly improved steatosis, inflammation, obesity, and insulin resistance [18, 19, 56, 57]. Furthermore, the inhibition of glutaminyl cyclases, an enzyme responsible for the maturation of cytokines to the active form, alleviates CCL2-mediated liver inflammation in an experimental model of NAFLD [58]. CCR5 antagonist, maraviroc, has been shown to be effective in the amelioration of NAFLD, indicating that CCR5 is also a promising therapeutic target for patients with NAFLD [59]. Of note, a CCR2/CCR5 dual antagonist, cenicriviroc, that was originally developed for the treatment of HIV infection is now in a phase 2 clinical trial for NASH-associated liver fibrosis in adult subjects [60]. Since CCR2 and CCR5 are important for the infiltration of both myeloid cells and hepatic stellate cell, we expect that this antagonist can suppress NAFLD development through inhibiting both inflammatory and fibrogenic pathways. Another study showed that pharmacological inhibition of CXCL16 reduced liver macrophage infiltration and steatohepatitis in the NASH mouse model [55]. Moreover, pharmacological inhibition of MLK3 prevented CXCL10 enrichment in hepatocyte-derived EVs and subsequently inhibited macrophage chemotaxis in the pathogenesis of NAFLD [43]. Of note, two recent studies suggest that β-cryptoxanthin protects and reverses NASH in mice through inhibition of lipid accumulation and lipid peroxidation by regulating the M1/M2 polarization of Kupffer cells in the liver. The mechanism of action is partly mediated through a downregulation of the CCL2/CCR2 and CCL5/CCR5 signaling [61, 62]. These previous findings and ongoing clinical trials suggest that targeting chemokines and chemokine receptors on inflammatory cells and hepatic stellate cells to control liver inflammation and fibrogenic response might represent promising therapeutic approaches for NAFLD and its related fibrosis.

4.9 Perspectives and Conclusions

Extensive in vitro and in vivo investigations conducted over the past 20 years have elucidated the pivotal roles played by the inflammation in the pathogenesis of NAFLD and fibrosis. It is becoming increasingly clear that chemokines and chemokine receptors play more important roles than we expected in the NAFLD development. Several chemokine systems may be integrally involved in tissue- and organ-level inflammation caused by interactions among liver, adipose tissue, and macrophages as well as the subsequent development of systemic insulin resistance and metabolic disorders. However, much research remains to be done to elucidate the pathophysiology of NAFLD and to identify specific targets for the treatment. Additionally, the involvement of chemokines and their receptors in the pathogenesis of NAFLD is still only partially understood.

Although the initial studies attempting therapeutic strategies targeting the chemokine system have been reported, further investigations of the underlying molecular mechanisms of NAFLD in which chemokine-chemokine receptor interactions play a role are indeed required. Collectively, all of the evidence supporting the mechanistic link between the chemokine-chemokine receptor system and NAFLD development provides important information for developing new options for the treatment of NAFLD, NASH, and fibrosis. Additional preclinical studies as well as clinical trials targeting chemokines and/or their receptors will provide better understandings of the underlying molecular mechanisms of chemokine system-mediated hepatic inflammation and the pathogenesis of NAFLD, which is crucial for developing novel treatments.

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