Molecular Pathways: Hepatitis C Virus, CXCL10, and the Inflammatory Road to Liver Cancer

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Abstract

An estimated 170 million people worldwide are chronically infected with the hepatitis C virus (HCV), which is characterized histologically by a persistent immune and inflammatory response that fails to clear HCV from hepatocytes. This response is recruited to the liver, in part, by the chemokine CXCL10, the serum and intrahepatic levels of which have been inversely linked to the outcome of interferon-based therapies for hepatitis C. Bystander tissue damage from this ineffective response is thought to lead to increased hepatocyte turnover and the development of fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). However, CXCL10 is traditionally viewed as an orchestrator of the angiostatic and antitumor immune response. In this review, we will explore this duality and the pathways by which CXCL10 is produced by hepatocytes during HCV infection, its effects on resident and infiltrating immune cells, and how deregulation of these cell populations within the liver may lead to chronic liver inflammation. We will also discuss potential host-directed therapies to slow or reverse HCV-induced inflammation that leads to fibrosis, cirrhosis, and HCCs.

Background

Chronic hepatitis C virus (HCV) infection affects an estimated 170 million people globally and is the leading cause of liver transplantation in many countries (1, 2). Activation of innate immune pathways in hepatocytes following infection leads to infiltration of proinflammatory, antiviral immune effector cells into the liver (3). Many of these cells are recruited to the liver by the chemokine CXCL10, which binds to and activates the CXCR3 receptor found most commonly on proinflammatory CD8+ cytotoxic T (Tc) cells, CD4+ type 1 helper T (Th1) cells, and natural killer (NK) cells (4, 5). However, this response is incapable of eliminating the virus in approximately 85% of patients with acute infection and instead contributes to a chronic immune cell presence in the liver (6). Indeed, CXCR3+ CD8+ Tc cells have been identified among intrahepatic immune cells in patients with chronic hepatitis C (4, 5). Damage to bystander tissue from this persistent yet ineffective inflammatory response has been linked to the development of fibrosis, cirrhosis, and hepatocellular carcinoma (HCC; ref. 7). CXCL10 plasma levels are also negatively correlated with the outcome of interferon (IFN)-based therapy for HCV infection (8). However, as an angiostatic chemokine that recruits CD8+ Tc and NK cells, CXCL10, could orchestrate an antitumor response (9). Herein, we will explore this apparent paradox by defining the innate immune signaling pathways that lead to CXCL10 induction in hepatocytes, examining how deregulation of the recruited immune response during HCV infection may lead to inflammatory liver disease, and discussing possible avenues for controlling inflammation and preventing the development of HCCs.

Innate Immune Sensing of HCV in Hepatocytes

Activation of cellular innate immune pathways depends upon recognition of foreign DNA, RNA, or protein motifs known as pathogen-associated molecular patterns (PAMP). Specific PAMPs are recognized by innate pattern recognition receptors (PRR) from 1 of 3 families: Toll-like receptors (TLR), retinoic acid inducible gene 1 (RIG-I)-like receptors (RLR), or Nod-like-receptors (NLR). The interplay of these receptors and their downstream signaling pathways is what determines the resultant innate immune response. For example, the positive sense HCV RNA genome is separately recognized by 2 different PRRs within the hepatocyte: RIG-I and TLR3 (Fig. 1; refs. 10, 11).

RIG-I is a cytoplasmic sensor of double-stranded, 5’ triphosphate RNAs containing poly-U or poly-A motifs (12). Following the binding of this PAMP, RIG-I undergoes a conformational change and binds to the mitochondrial antiviral-signaling protein (MAVS) signaling adaptor (13). In contrast, TLR3 recognizes longer double-stranded RNAs generated during viral replication that have been relocalized to the endosome (11). Activated TLR3 binds the signaling adaptor TRIF domain-containing adapter-inducing IFN-β (TRIF) through its cytoplasmic receptor domain (13).
Induction of CXCL10 in Hepatocytes

MAVS and TRIF signaling activates various transcription factors including NF-κB, activator protein (AP)-1, C/EBP-β, and IFN-regulatory factors (IRF), which translocate into the nucleus to induce gene transcription. Putative binding sites for these transcription factors have been annotated in the CXCL10 promoter. Indeed, HCV can induce NF-κB binding to this site in TLR3-expressing hepatoma cells. NF-κB also drives CXCL10 transcription during rhinovirus infection, whereas AP-1 and C/EBP-β activate transcription of the structurally similar chemokine CXCL8 [i.e., interleukin (IL)-8; refs. 15–17]. IRF1, IRF2, IRF3, and IRF7 also reportedly bind the CXCL10 promoter during influenza A infection.

Activation of IRF3 and IRF7 can also lead to the induction of antiviral type I IFNs (IFN-α and IFN-β) and type III IFNs (IL-28A, IL-28B, IL-29) in hepatocytes. These secreted cytokines can act in a paracrine manner to amplify chemokine and cytokine responses in adjacent liver cells through activation of Janus kinases (JAK) and various STAT proteins. Over several decades, progressive fibrosis can lead to cirrhosis and HCCs.

Figure 1. Deregulation of the inflammatory response recruited by CXCL10 following HCV infection. Sensing of viral RNA by the innate immune receptors RIG-I and TLR3 following hepatitis C virus (HCV) infection of the hepatocyte leads to signal transduction through MAVS and TRIF, respectively, activation of transcription factors (NF-κB, IRFs, AP-1, C/EBP-β), and transcription of CXCL10 (Δ; “CXCL10 Induction”). Secreted CXCL10 forms a chemotactic gradient that recruits immune cells (NK, CD4+ TH1, and CD8+ Tc cells) and nonparenchymal liver cells (Kupffer cells and HSCs) to the site of infection (“Recruitment, Inflammation, and Cell Death”). Upon arriving, these cells produce proinflammatory, proapoptotic mediators (ϕ) such as type I IFN, type III IFN, TNFα, IL-1β, and ROS. This response fails to clear HCV in 80% to 85% of patients and instead generates persistent inflammation and hepatocyte turnover. It also leads to liver fibrosis through chronic HSC activation, the overproduction of type I collagen, and the inhibition of collagen-degrading MMPs by TIMPs (“Cell Turnover and Fibrosis”). Over several decades, progressive fibrosis can lead to cirrhosis and HCCs.
NK cells, CD8+ Tc cells, and CD4+ Tg1 cells, can also induce STAT1 signaling through these elements (20, 21). As the CXCL10 promoter contains both putative ISREs and putative STAT-binding sites, it can potentially respond to all 3 types of IFN (15).

Despite these observations in other systems, we observed that neutralization of type I and type III IFNs had no effect on CXCL10 production during HCV infection in hepatoma cells expressing functional TLR3 and RIG-I (22). These data suggest that CXCL10 induction in hepatocytes during the initial steps of HCV infection occurs predominantly through direct activation of transcription factors following PRR signaling rather than through secondary paracrine signaling of IFNs. Of course, IFNs secreted from immune cells recruited to the HCV-infected liver as well as from nonparenchymal cells likely contribute to CXCL10 induction in vivo. This secondary induction would supplement the initial CXCL10 output by hepatocytes.

Induction of CXCL10 in hepatocytes may also involve nontraditional PRR signaling pathways. Ho and colleagues reported IFN-independent activation of STAT1 and STAT3 proteins during infection with dengue virus, another member of the Flaviviridae (23). STAT1 can also be activated via p38 mitogen-activated protein kinase (MAPK) following TLR7 stimulation in plasmacytoid dendritic cells (24). As STAT1 can bind to ISREs, it is possible that this alternative pathway contributes to CXCL10 induction in hepatocytes.

**CXCL10 Recruits Proinflammatory Effector Cells for the Anti-HCV Response**

Once induced, CXCL10 recruits a proinflammatory, antiviral immune response to sites of infection by binding to the CXCR3 receptor on CD4+ Tg1 and CD8+ Tc cells (Fig. 1; refs. 4, 5). CXCR3 was recently reported to be universally expressed and exists in 2 isoforms: CXCR3A and CXCR3B (25). CXCR3A is the activating isoform highly expressed by leukocytes and is associated with proliferation and chemotactic migration of these cells (25, 26). CXCR3 is also expressed by NK cells as well as by minority cell populations within the liver including resident macrophages (i.e., Kupffer cells) and hepatic stellate cells (HSC; refs. 4, 27–29). Thus, CXCL10 induction from hepatocytes could also localize nonparenchymal cells within the liver to specific sites of infection.

Once recruited to the inflamed liver, activated CD8+ Tc and NK cells kill virus-infected cells via Fas/TRAIL-mediated apoptosis, the release of granzymes and perforin, and secretion of type II IFN (27, 30). Apoptotic bodies released from dying hepatocytes are then phagocytosed by Kupffer cells, which further promote Fas-mediated hepatocyte apoptosis and release reactive oxygen and nitrogen species (ROS/NOS; ref. 31). Kupffer cells also activate HSCs by releasing TGF-β (31). This causes HSCs to differentiate from quiescent vitamin A storage bodies into proliferative myofibroblasts that secrete type I collagen as part of the general wound-healing response to liver injury (32).

Kupffer cells, HSCs, and liver sinusoidal endothelial cells (LSEC) also perpetuate the existing inflammatory state by secreting additional cytokines and chemokines as part of a positive feedback loop. As in hepatocytes, this secretion can be triggered by proinflammatory cytokines produced by infiltrating immune cells (TNFα, IFNs, etc.) or by innate PRRs. Recognition of HCV nonstructural proteins by TLR4 in Kupffer cells during chronic infection can increase secretion of TNFα (33). TNFα- and IL-1β-activated HSCs show increased secretion of CXCL8 when exposed to ligands for TLR2, which recognizes HCV core and NS3 proteins (34, 35). Supernatants from LSECs treated with TLR3- and TLR4-specific PAMPs were also able to suppress HCV replication in HCV replicon-bearing cells (36). Thus, the primary sensing of HCV RNA by hepatocytes initiates an antiviral, proinflammatory response that involves recruitment of multiple immune cell types to the liver that further amplifies the response.

**Deregulation of Recruited Cells Leads to Fibrosis, Cirrhosis, and HCC**

Despite the robust inflammatory response initiated and recruited by CXCL10, chronic hepatitis C develops in up to 85% of subjects with acute infection (6). Viral evolution plays a considerable role in establishing this persistence, as immune escape variants of the HCV NS3 epitope recognized by CD4+ Tg1 cells fail to stimulate proliferation while simultaneously causing these cells to shift to a Tg12 response profile (37). This causes induction of anti-inflammatory cytokines (i.e., IL-10) and reduction of CD8+ Tc and NK cell–stimulating cytokines (i.e., type II IFN and IL-2; ref. 38). Direct inactivation of infiltrating effector cells can also lead to ineffective viral clearance. HCV-specific CD8+ Tc cells from patients with chronic hepatitis C display an exhausted phenotype, with decreases in both type II IFN production and epitope-specific degranulation (39). Virus-mediated dendritic cell dysfunction may contribute to the development of anergy through ineffective costimulation or antigen presentation, as could the presence of an antagonistic variant of CXCL10 which may inhibit migration of these CXCR3+ cells from plasma into tissue (40, 41). Higher frequencies of both intrahepatic and peripheral CD4+ CD25+ FoxP3+ immunosuppressive regulatory T (Treg) cells have also been reported in HCV-infected patients, further indicating that suppression of effector immune responses maintains viral persistence in chronic hepatitis C (40, 42).

HCV proteins also interfere with antiviral and IFN responses in hepatocytes during chronic infection (43, 44). Despite this interference, elevated levels of inflammatory cytokines and chemokines are still found in the liver parenchyma of patients with chronic hepatitis C (see above). Kupffer cells also remain activated and continue to release ROS/NOS and TGF-β, perpetuating HSC activation and type I collagen deposition. Eventually, chronic activation causes HSCs to secrete tissue inhibitor of metalloproteinases (TIMP), which inhibit collagen-degrading matrix metalloproteinases (MMP) and lead to an excessive accumulation of fibrotic scar tissue known as fibrosis (32).
Progressive disruption of the liver architecture and continued hepatocyte turnover can then lead to cirrhosis, a condition in which the liver parenchyma is divided into isolated nodules of regenerative tissue with severely reduced functionality (31). Accumulation of genetic aberrations from repeated rounds of cell death and renewal within these nodules then leads to neoplasm and HCCs (7).

The proinflammatory and cytotoxic immune responses recruited by CXCL10 can normally eliminate precancerous and cancerous cells through recognition of tumor-specific antigens (7). However, as these responses are already impaired during chronic hepatitis C, it is likely that the ability to identify and eliminate neoplastic cells is also defective. CXCL10 may still inhibit development of HCCs through its reported angiostatic activity, but recent literature suggests that CXCL10 may accelerate cancer growth in non-immune cell types (45, 46). Neoplastic cells may also exploit chemokine gradients as “roads” during metastasis. Treatment with CXCL10 increases motility of prostate cancer-derived but not normal prostate epithelial cells via reduced CXCXR3B expression, which normally inhibits cell growth and migration in nonmotile cell types (25, 47). CXCXR3B expression was also reduced in 2 breast cancer cell lines, whereas induction of CXCXR3A and repression of CXCXR3B have been reported in clear cell ovarian cancers (48, 49). It remains to be determined whether downregulation of growth-inhibitory receptor CXCXR3B and/or upregulation of the growth-promoting receptor CXCXR3A occurs during hepatocyte transformation to HCCs and metastasis.

Clinical–Translational Advances

Current therapies for chronic hepatitis C seek to limit the development of persistent inflammation by reducing systemic viral load using a combination treatment of PEGylated IFN-α and the nonspecific antiviral Ribavirin (peg-IFNα/RBV). Unfortunately, this regimen fails to eliminate the infection in roughly 50% of patients (6). While recently developed HCV-specific protease inhibitors improve the likelihood of success for some patients, IFN-containing regimens are still poorly tolerated, require 24 to 48 weeks of administration, and do not address the underlying inflammatory sequelae that cause liver disease (50, 51). For patients who have already progressed to decompensated cirrhosis, liver transplantation represents the only available treatment option (52). However, re-infection of the new liver occurs in nearly all cases of active infection, and anti-HCV therapy is less efficacious and associated with increased toxicity after transplantation (52). Thus, new treatments that prevent or reverse the onset of these inflammatory sequelae must be pursued. As a master regulator of the infiltrating proinflammatory response, the CXCL10/CXCR3 signaling pathway makes an attractive therapeutic target.

Potential Anti-CXCL10 Therapies

Agents that selectively neutralize CXCL10 would theoretically increase patient responsiveness to traditional IFN-based HCV therapy while simultaneously dampening inflammatory immune cell activation. For example, specific inhibitors of the CXCR3A isotype could prevent aberrant activation of CD8+ T cells and NK cells that lead to excessive hepatocyte death. This, in turn, would limit Kupffer cell and HSC activation and delay or prevent development of fibrosis. Such drugs would likely mimic Maraviroc, an antagonist of the chemokine receptor CCR5 that is used clinically to block HIV entry (53). However, it is possible that reducing a patient’s sensitivity to CXCL10 by blocking its receptor may also interfere with the immune system’s ability to respond to other pathogens (54).

Broadly Acting Anti-inflammatory Therapies

A safer alternative may be to identify new applications for existing anti-inflammatory drugs. One advantage to this approach is the ability to counteract the excessive immune response recruited by CXCL10 through multiple mechanisms. For example, as oxidative stress causes direct cellular damage in addition to activating HSCs, herbal antioxidants compounds have been suggested as both antiinfectious and anti-inflammatory therapy for liver diseases of multiple etiologies (31). Vitamin E has successfully reduced inflammation and halted fibrosis progression among those with nonalcoholic steatohepatitis (NASH) in clinical trials (55). The routinely consumed herbal medications Sho-saiko-to and Silymarin also appear to have direct antiinflammatory activity on HSCs as well as general hepatoprotective properties, although their mechanisms of action remain undefined (56, 57). Traditional antifibrotic drugs have also had demonstrable effects on oxidative stress within the liver: long-term treatment with Losartan reduces NADPH oxidase activity in patients with HCV (58).

Successful anti-inflammatory therapies may also target pathways other than those involved in generating oxidative stress. Broadly acting corticosteroids remain a standard therapy for autoimmune hepatitis (59). Sorafenib, a chemotherapeutic agent already approved to treat HCCs, also inhibits the Raf/ERK proinflammatory and profibrotic signaling pathways (60). Finally, TNFα inhibitors have been used reduce serum levels of liver enzymes, IL-6, and TGF-β in animal models, although limited success has been seen in human clinical trials for alcohol-related liver disease or advanced cirrhosis (31).

Targeting multiple pathways simultaneously may also increase the risk of adverse events occurring during treatment. Severe side effects have been reported among patients taking experimental broadly antiapoptotic drugs such as caspase-3 inhibitors (31). The duration of anti-inflammatory therapy will also likely depend upon the extent of fibrosis or cirrhosis present within the liver, increasing the likelihood of adverse events occurring in patients with severe disease. In addition, administering anti-inflammatory drugs to patients simultaneously undergoing IFN treatment for hepatitis C may interfere with the antiviral efficacy of IFN.

Ultimately, a better understanding of immune and inflammatory signaling within the liver is required before
the full extent of the efficacy and side effects for these proposed treatments can be known. As HCV-related cirrhosis and HCCs are predicted to increase substantially in the next decade (61), it is imperative that research into this area accelerates. Routine clinical application of hepatoprotective therapies in the near future may help to prevent or reverse the effects of end-stage liver disease in millions of chronically infected patients with HCV worldwide. Furthermore, these types of host-directed therapies may be beneficial to other, nonviral forms of liver diseases that include an inflammatory component.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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