Hepatic Stellate Cell-Immune Interactions in NASH

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Nonalcoholic fatty liver disease (NAFLD) is the dominant cause of liver disease worldwide. Nonalcoholic steatohepatitis (NASH), a more aggressive presentation of NAFLD, is characterized by severe hepatocellular injury, inflammation, and fibrosis. Chronic inflammation and heightened immune cell activity have emerged as hallmark features of NASH and key drivers of fibrosis through the activation of hepatic stellate cells (HSCs). Recent advances in our understanding of the molecular and cellular pathways in NASH have highlighted extensive crosstalk between HSCs and hepatic immune populations that strongly influence disease activity. Here, we review these findings, emphasizing the roles of HSCs in liver immunity and inflammation, key cell-cell interactions, and exciting areas for future investigation.

Keywords: hepatic stellate cells, NASH, inflammation, fibrosis, immunity

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the leading cause of liver disease globally and is projected to overtake hepatitis C as the primary indication for liver transplantation in the US and Europe, along with alcoholic liver disease (1, 2). NAFLD comprises a spectrum of liver pathology from simple steatosis with increased hepatocyte lipid content but no inflammation, termed non-alcoholic fatty liver (NAFL), to non-alcoholic steatohepatitis (NASH) characterized by hepatocyte death, inflammation, and fibrosis. NASH affects roughly 1 in 5 patients with NAFLD, conferring a sizable risk of cirrhosis and hepatocellular carcinoma (HCC) (3).

Sustained inflammation is thought to drive the transition from simple steatosis to NASH (4, 5). An important downstream consequence of hepatic inflammation is the activation of hepatic stellate cells (HSCs), the principal fibrogenic cell type in the liver (Figure 1). Fibrosis severity mirrors disease progression and is the only histologic feature that predicts liver-related mortality in NASH patients (6). Interplay between HSCs and hepatic immune cells, long recognized as a key feature of the hepatic injury response, has emerged as an especially important determinant of NASH pathogenesis (5, 7, 8). This review highlights the roles of HSCs in hepatic immunity and their impact in NASH.
INFLAMMATION AND IMMUNE CELL ACTIVATION IN NASH

Inflammation in NAFLD is triggered by a wide range of insults that precipitate hepatocyte injury and death, and increase systemic inflammatory signals. The disease drivers in NAFLD are numerous and interdependent. Among the most significant are altered hepatocyte metabolism, lipotoxicity, and oxidative stress, which lead to endoplasmic reticulum stress and mitochondrial dysfunction. Additional dysregulation of liver homeostasis results from visceral fat inflammation, peripheral insulin resistance, and disruptions to enterohepatic bile acid circulation (3, 9). Hepatocyte-derived extracellular vesicles (EVs) carrying chemokines, inflammatory mediators, and fibrogenic micro-RNA (miR-128-3p) are increased in NASH and implicated in myeloid and HSC activation (10). Collectively, these changes lead to hepatocellular injury and increased damage- and pathogen-associated molecular patterns (DAMPs and PAMPs).

Murine models and human NASH biopsies show hepatocytes activating multiple pathways of programmed cell death. There is an increasing appreciation that lytic cell death programs, including necrosis, necroptosis, and pyroptosis are important inflammatory drivers in the pathogenesis of NAFLD (11). These pathways involve rapid membrane permeabilization and release of cytoplasmic contents, triggering a stronger immune response than apoptosis (12).

Disturbance of the gut-liver axis due to NAFLD-associated dysbiosis has also emerged as an important extrahepatic source of inflammation in both experimental models and human NAFLD cohorts (13). Although disease-associated microbes vary widely between studies, the impact of changes to the composition and function of the microbiome in NASH include altered bile acid metabolism and loss of integrity of the gut-vascular barrier, precipitating leakage of LPS and bacteria into the liver via the portal circulation (14–16).

The liver’s rich community of innate and adaptive immune cells undergoes dramatic remodeling during the pathogenesis of NASH, with an overall increase in immune cell infiltration. Early inflammatory signals stimulate recruitment of neutrophils and accumulation of monocyte-derived macrophages (MoMFs), adding to the large liver-resident Kupffer cell (KC) population already present (17). Activated dendritic cells (DCs) increase in abundance, coordinating the adaptive immune response as professional antigen presenting cells (APCs) (18). Lymphocytes, including conventional CD4+ and CD8+ T cells, release HSC-activating cytokines like TNFα and IL-6. Natural killer T (NKT) cells promote HSC activation through release of osteopontin (Opr) and sonic hedgehog (Shh). Natural killer (NK) cells display reduced HSC killing due to increased insulin and TGFβ signaling.
unprecedented view of cellular heterogeneity and transcriptional activity in the liver, and is critical to building a global understanding of the key cell-cell interactions driving disease (19–22).

HEPATIC STELLATE CELLS

Hepatic stellate cells (HSCs) are a versatile mesenchymal cell type with wide ranging roles in liver development, hepatocyte homeostasis, retinoid storage, and the liver’s coordinated response to injury (23). Stellate cells line the space of Disse, a niche in between sinusoidal endothelial cells and the basolateral surface of hepatocytes, establishing a central vantage point from which they monitor the hepatic environment for pathogens and hepatocellular damage. Upon liver injury, HSCs activate and transdifferentiate to generate an expanded population of myofibroblasts that are inflammatory, contractile, and produce large quantities of extracellular matrix (ECM) (24). HSC activation is an adaptive response that helps the liver respond to frequent exposure to pathogens and toxins; however, ongoing activation due to sustained liver injury leads to excess ECM deposition (ie. liver scar or hepatic fibrosis), the hallmark of chronic liver diseases including NASH (24).

HSC activation is driven by many signaling molecules and convergent pathways that conspire to initiate, and then perpetuate, the transition to a fibrogenic myofibroblast program. There are several excellent reviews of the numerous pathways involved in HSC activation including fibrogenic, proliferative, and inflammatory cytokines, Hedgehog signaling, metabolic reprogramming, cholesterol signaling, and oxidative stress (24–26). Among the most potent activating signals are cytokines and growth factors secreted by hepatic immune cells. Direct activation of HSC-expressed innate immune receptors, including the Toll-like receptors (TLRs) and complement pathway, provide additional direct activating stimuli to HSCs (8, 24). Although the primary focus thus far in studying HSC-immune crosstalk has been on its contribution to HSC activation, these interactions are bidirectional and also include contributions by HSCs as an important effector cell of the hepatic immune system. In view of the prominent role played by hepatic immune cells in NASH, HSC-immune communication is a growing area of interest (5).

INTRODUCTION TO HEPATIC STELLATE CELL IMMUNE FUNCTIONS

HSCs are deeply integrated into the hepatic immune system. They contribute directly to the liver’s robust and essential innate immunity through expression of a large repertoire of innate immune receptors (8, 27). In doing so they amplify pro-inflammatory cytokine production, recruit monocytes and lymphocytes to the liver, and engage directly with other effector cells of the immune system. This tight intercellular communication is ultimately reflected in the coordination between HSC activation state and hepatic immune tone (7, 8, 27, 28).

Innate Immune Receptors

The liver is the first line of defense against invading pathogens, bacterial metabolites, and toxins entering the portal circulation from the gut. To protect against these threats, the liver recruits innate immune cells and also engages other liver cell populations including liver sinusoidal endothelial cells (LSECs), Kupffer cells (KCs), and HSCs (27).

Quiescent HSCs (qHSCs) are primed by expression of multiple pattern recognition receptors (PRRs), including TLRs 3 and 4 (29). TLR3 stimulation by double stranded RNA in qHSCs prompts IFNγ production (29). Activated HSCs (aHSCs) express an even larger set of TLRs (TLR2, TLR3, TLR4, TLR7, and TLR9) (8). The role of TLR4 and its ligand, LPS, is best characterized. When exposed to low levels of LPS, aHSCs activate the NF-κB pathway, secrete pro-inflammatory cytokines including IL-8, upregulate leukocyte adhesion molecules ICAM-1 and VCAM-1 and suppress the TGFβ pseudoreceptor Bambi (30, 31). Polymorphisms that attenuate HSC TLR4 activity reduce inflammatory signaling and are protective against fibrosis (32). TLR9 binds DNA released by apoptotic hepatocytes, stimulating fibrogenic pathways in aHSCs and halting their migration at injury sites (33).

Immunoregulatory Functions

Beyond their roles in innate immunity, HSCs interact extensively with infiltrating immune populations and directly regulate their behavior. Activated HSCs recruit immune cells by secreting numerous chemokines including monocyte chemoattractant protein-1 (MCP-1) (34), IL-8 (35), RANTES and CCR5 (36), and SDF-1/CXCL-12 (37) and express adhesion molecules, ICAM-1 and VCAM-1, that promote the infiltration of leukocytes and macrophages (38, 39). HSCs can acquire some features of antigen presenting cells (APCs), including expression of major histocompatibility complexes (MHC) class I and II, and T cell-activating costimulatory molecules such as CD86 (40–42), although more recent evidence suggests HSCs have limited APC capabilities in vivo (43).

Surprisingly, several mechanisms have been identified by which activated HSCs are capable of promoting a tolerogenic environment in the liver. In co-culture experiments, HSCs interfere with T cell priming by dendritic cells (DCs) via a CD54 (ICAM-1)-dependent signaling pathway and by inducing STAT3 signaling in DCs (44, 45). HSCs cull the pool of activated T cells by expressing PD-L1 to induce T cell apoptosis (46–48). Remarkably, HSCs may oppose B cell activity via the same mechanism (49). They also amplify populations of tolerizing immune cells including FoxP3+ regulatory T cells (via retinoic acid and TGFβ signaling) (41, 43, 45, 50, 51) and myeloid-derived suppressor cells (52). Supporting these physiologic roles in promoting hepatic immune tolerance are studies in which HSCs are deleted, leading to increases in the numbers of CD4+, CD8+ T cells, DCs, natural killer (NK) cells, regulatory T cells, and Ly-6C+ macrophages (53). The practical impact of these proposed tolerogenic roles for aHSC, which
would appear to conflict with their inflammatory properties, remains uncertain. In chronic liver disease, the balance of HSC-immune interaction favors inflammatory, pro-fibrogenic signaling; however, it is possible that a minority population of tolerogenic HSCs may be identified in future studies.

HSC – Immune Cell Interactions
From their perch along fenestrated LSECs in the perisinusoidal space, HSCs communicate directly with both resident liver cell populations as well as infiltrating immune cells. Recent single cell RNA sequencing data from the liver have reinforced the HSC’s role as a key signaling hub with many immune cell communications. Algorithms that analyze ligand-receptor pairs to predict cell-cell signaling have demonstrated that HSCs have among the greatest number and most diverse interactions of any hepatic cell type including with KCs, MoMF and to a lesser extent T, B, and NKT cells (19, 54). The number of predicted HSC-cell interactions is greatly increased upon HSC activation, highlighting the importance of these contacts during disease progression (54).

HSC-immune crosstalk is most clearly illustrated by interactions with macrophages. Upon liver injury, signaling between HSCs and macrophages coordinates the activation of both populations. Macrophages are the main cellular source of potent pro-fibrogenic signals including TGFβ, PDGF, TNF, and IL-1β, and also secrete matrix metalloproteinases (MMPs) that activate latent TGFβ stored in the ECM (24, 55). ScRNAseq of human cirrhotic livers has identified a subset of TREM2+CD9+ macrophages enriched in heavily scarred regions of the liver that secrete cytokines and growth factors, including epidermal growth factor (EGF) and platelet-derived growth factor BB (PDGF-BB) to create a pro-fibrogenic niche for aHSCs (56). HSCs, in a reciprocal fashion, can amplify the fibrogenic activity of macrophages. In a co-culture system aHSCs induced a pro-inflammatory profile in macrophages through a p38-dependent signaling pathway (57). Even so, parallel lines of communication between HSCs and macrophages appear to limit the extent of liver damage. HSCs release signals such as CX3CL1 that restrain pro-fibrogenic signaling by macrophages even during ongoing injury (58, 59). Moreover, infusion of bone marrow-derived macrophages in mice reduces fibrogenesis via increased matrix-degrading MMPs and anti-inflammatory IL-10 (60). These effects are likely due to the presence of “restorative macrophages” characterized by low expression of Ly-6C (in mice), elevated matrix remodeling enzymes and phagocytosis of apoptotic bodies (61, 62). The delicate balance between HSCs and macrophages has recently been distilled into a model of paracrine signaling between the two cell types that produces a stable two-cell circuit which largely predicts their behaviors under healthy and disease conditions (63, 64).

Interactions between HSCs and other immune populations are less extensively catalogued but important. HSC activation in experimental fibrosis models is reduced in immunodeficient (SCID) mice and rescued by adoptive transfer of lymphocytes, especially CD8+ T cells (65). Similarly, B cells and HSCs form a pro-fibrogenic network in mice subjected to CCl4 liver injury. Retinoic acid (RA) signaling by HSCs promotes B cell survival and activation while, in turn, B cells secrete inflammatory cytokines (66). Retinoic acid signaling has emerged as an important immunomodulatory mediator, particularly in promoting Th17 cell differentiation. RA receptor (RAR) synthetic agonists and all-trans retinoic acid (ATRA) have also shown direct anti-fibrotic effects on HSCs (67, 68).

In other contexts, immune cells may reign in HSC fibrogenic responses. Neutrophils, signaling to macrophages, facilitate the switch from disease progression to resolution – a phase when liver injury has ceased and there is a global shift away from inflammatory signaling and toward repair, including fibrosis regression (69). Similarly, interferon-γ (IFNγ), produced by many immune cells including NK, NKT and T cells, has direct anti-fibrogenic activity on HSCs (70–72).

HSCS AND IMMUNITY IN NASH
The ascendance of NASH as a worldwide health concern has spurred investigations into the immunologic pathways responsible for disease progression. These efforts have been aided by the development of murine models that capture many of the key histologic and transcriptomic features of human NASH, and by the application of single cell RNA sequencing to the liver (24, 73). The sections below highlight recent advances and summarize the pathways by which HSCs communicate with the immune system in NASH.

Innate Immunity
Innate immune activation is an important fibrogenic stimulus in NASH (3). While it is beyond the scope of this work to provide a comprehensive review of innate immunity in NASH [see (9, 74)], the prominent contributions of inflammasome activation and changes to the microbiome are highlighted here (Figure 1).

Inflammasomes are multiprotein innate immune receptors present in the cytoplasm of hepatocytes and nonparenchymal liver cells. Upon stimulation by hepatocellular injury or pathogens, inflammasomes activate caspase-1 triggering release of inflammatory cytokines IL-1β and IL-18 and programmed cell death (9). The NLRP3 inflammasome has emerged as a key mediator of the transition from steatosis to NASH. In addition to the propagation of hepatic inflammation, the NLRP3 inflammasome can activate HSCs directly. Selective expression of a constitutively active transgenic NLRP3 in mouse HSCs is sufficient to induce fibrosis (75). Additionally, activated NLRP3 particles released by hepatocytes undergoing pyroptosis are endocytosed by HSCs, triggering activation and increased IL-1β production (76). Blockade of NLRP3 signaling with a small molecule inhibitor reduces disease severity in the MCD – Foz/Foz murine NASH model, although the relative contributions of targeting other inflammasome-expressing populations, including myeloid cells, have not been determined (77).

NAFLD-related changes to the composition of the gut microbiome and impaired intestinal barrier function flood the liver with PAMPs including the potent TLR4 ligand, LPS (78). These changes are positively correlated with NASH severity and
fibrosis in patient cohorts and experimental NASH models (16, 79–82). Restoration of gut barrier integrity in a high fat diet (HFD) mouse model of NASH dampens innate immune signaling and reduces HSC activation (16).

Macrophages

Macrophages in the healthy liver are made up of KCs and circulating MoMFs. KCs maintain normal liver homeostasis as an important phagocytic cell type and essential component of the sinusoidal niche, but are stimulated by injury signals in NAFLD, prompting them to adopt an activated expression profile and recruit large numbers of inflammatory Ly-6C\(^{hi}\) MoMFs. This inflammatory switch is one of the definitive steps in the transition from NAFLD to NASH and the progression of fibrosis (4). Sustained disease drives a shift towards “alternatively activated M2-type” macrophages, characterized by heightened fibrogenic signaling (83).

Macrophages respond to a unique cocktail of injury signals in NASH, including signs of hepatocyte injury, cholesterol and lipid metabolites, and LPS from the “leaky gut” caused by NAFLD-associated dysbiosis (84). Circulating mitochondrial DNA is a powerful inflammatory signal in NASH that activates the antiviral response molecule, STimulator of Interferon Genes (STING), in macrophages (85, 86). In response to these stimuli, macrophages secrete cytokines, chemokines, and other soluble signals that contribute to fibrosis by: (A) driving HSC activation through release of TGF\(\beta\), PDGF, TNF, FGF2, MCP1, CCL3, CCL5 and reactive oxygen species (ROS), and; (B) promoting aHSC survival by activating the NFkB pathway with IL-1 and TNF (24). Not surprisingly, depleting hepatic macrophages in mouse models with agents such as clodronate liposomes, or blocking their recruitment pharmacologically, as with the CCR2/CCR5 antagonist Cenicriviroc, attenuate fibrosis and blunt other histologic markers of disease (87–91). PPAR\(\delta\) agonism can modulate MoMF gene expression to improve lipid handling and decrease pro-fibrogenic signaling to HSCs, synergizing with pan-PPAR agonism as a potential NASH therapy (92).

Recent studies have revealed how the hepatic macrophage population changes in response to NASH, with implications for their interactions with HSCs. A TREM2\(^{+}\) macrophage subtype that is strongly linked to markers of tissue injury and fibrosis is enriched in NASH livers (19). The role of TREM2\(^{+}\) macrophages in NASH is unsettled, and recent evidence suggests they may contribute to an adaptive response to metabolic injury in NASH and promote fibrosis regression (93, 94).

The pool of resident KCs undergoes maladaptive changes in NASH. This was highlighted by two recent studies that used mouse models of NASH to explore changes to hepatic macrophages during disease progression (20, 95). Using a combination of KC-specific markers, parabiosis studies, and bone marrow transplant experiments, they demonstrated that liver resident TIM4\(^{+}\) KCs die and are replaced by a KC-like population derived from Ly-6C\(^{hi}\) MoMFs that are more inflammatory than their predecessors. Intriguingly, HSCs, along with LSECs and hepatocytes, provide niche cues that recruit MoMFs and instruct their differentiation to the KCs fate (96). This finding raises the prospect that HSCs contribute to the acquisition of a more inflammatory and pro-fibrogenic KC population in NASH. Those MoMFs not fated to become KCs remain in the liver as CLEC4F\(^{+}\) SPPI\(^{+}\) TREM2\(^{+}\) CD9\(^{+}\) “Lipid-Associated Macrophages” (LAMs) with more activated transcriptional profiles. This population was found to associate closely with aHSCs and likely overlaps with the scar-associated TREM2\(^{+}\) CD9\(^{+}\) macrophages described by Ramachandran et al. in scRNAseq of human cirrhosis (20, 56). Finally, studies of NASH histology have identified rings of macrophages forming “Crown-like Structures” (hCLS) around dying hepatocytes that were initially thought to represent a more inflammatory macrophage subset (97, 98). Interestingly, reduced hCLS formation in Ccr2 KO mice fed a HFD was associated with similar weight gain and steatosis, but increased fibrosis, suggesting a protective role for macrophages in this context (99).

Extensive cell-cell communications between HSCs and macrophages in NASH is an area of exciting new investigation. Analysis of receptor ligand pairs using scRNAseq from the AMLN murine NASH model identified a set of HSC-specific secreted factors termed “stellakines” and established HSCs as a signaling hub that interacts extensively with LSECs, macrophages, and to a lesser extent, DCs, T cells, and B cells. The immune-targeting “stellakines”, which are upregulated in NASH, included CCL2, CCL11, CXCL10, CXCL12, CXCL16, CTGF, and Gas6 (19).

Further evidence for HSC-macrophage crosstalk emerged in a characterization of the MerTK receptor. MerTK is a surface receptor predominantly expressed by macrophages that binds several ligands including the “stellakine” Gas6. When activated it induces TGF\(\beta\) production. ADAM17 cleaves MerTK to control macrophage inflammatory responses in steatotic livers, but this compensation fails in NASH – possibly due to reduced availability of HSC-supplied vitamin A (which is depleted when HSCs activate) that is necessary to stimulate ADAM17 (100).

While early studies of macrophage depletion in the CCl4 mouse model of liver fibrosis characterized a Ly-6C\(^{lo}\) restorative macrophage subset, additional studies are needed to clarify how macrophages specifically contribute to disease resolution in NASH (61). Emerging evidence points to Specialized Poresolving Mediators (SPMs) including marisens and resolvins as important players. At least some of these lipid metabolites are produced by macrophages (101) and also signal to macrophages to reduce inflammatory and fibrogenic gene expression (102). Other pathways to limit macrophage-HSC fibrogenic signaling are likely awaiting discovery. For example, investigators used a novel metabolomic and stable isotope tracing approach to uncover a hepatocyte-macrophage acetooacetate exchange that blocks fibrogenic signaling to HSCs in a HFD mouse model, although the detailed HSC-macrophage signaling pathways remain uncertain (103).

Neutrophils

As first responders in innate immunity, neutrophils amplify early inflammatory insults in NAFLD. Accordingly, depleting or
impairing neutrophils is protective in experimental NASH models (104). HSCs are primarily activated downstream of liver injury mediated by inflammatory and oxidative neutrophil effector functions. Release of myeloperoxidase, an enzyme that catalyzes the production of multiple oxidant species, is particularly harmful in the MCD NASH model. In humans, hepatic MPO content is positively correlated with NASH severity (105, 106). MPO is also directly stimulatory towards cultured HSCs and can activate latent TGFβ in liver homogenates, pointing to pathways of direct HSC activation by neutrophils (105). During inflammatory injury, HSCs may form a positive feedback loop with neutrophils by secreting factors such as GM-CSF and IL-15 that extend the neutrophil half-life, at least in coculture experiments (107). As observed with other immune cell types, neutrophils may have dual functions in NASH depending on disease stage. Depletion of neutrophils after establishment of liver injury and during the resolution phase of disease impairs fibrosis regression in the MCD NASH model, where neutrophils may be an essential source of miR-223, a microRNA that suppresses the NLRP3 inflammasome in macrophages (69) (Figure 2).

**NK Cells**

Early studies in experimental liver fibrosis indicated that NK cells were programmed to selectively target and kill activated HSCs, raising hopes that NK cells could have fibrolytic activity in chronic liver diseases (108–111). Activated NK cells also secrete antifibrotic IFNγ, although in advanced fibrosis HSCs inhibit NK cells through release of TGF-β (Jeong Hepatology 2011). In NASH, increased recruitment of NK cells to the liver is correlated with more severe disease (112). Part of the reason NK cells fail to control fibrogenesis in NASH may be that their HSC-targeting activity is being inhibited. NK cells isolated from a cohort of patients with NAFLD-NASH displayed abrogated HSC killing when derived from individuals with more severe insulin resistance and advanced fibrosis (113). In a murine NAFLD model, increased TGFβ signaling led to loss of NK cell cytolytic activity – potentially an adaptive mechanism to reduce liver inflammation (114). Together, these studies suggest that worsening insulin resistance and fibrogenic TGFβ signaling during NASH progression may blunt anti-myofibroblast surveillance by NK cells.

**NKT Cells**

NKT cells are the main unconventional innate-like T cells in the liver, along with γδ T cells and mucosal-associated invariant T (MAIT) cells (115). NKT cells have emerged as key drivers of hepatocyte injury during NASH pathogenesis, but the significance of direct NKT-HSC interactions is not well characterized (115, 116). NKT cells secrete HSC activating ligands sonic hedgehog (Shh) and osteopontin (Opn) in the MCD mouse model of NASH (117–120). Although other hepatic cell types produce Opn, NKT-deficient Jot18−/− and CD1d−/− transgenic mice have lower total hepatic Opn levels and reduced fibrosis. Moreover, NKT conditioned medium stimulates HSC activation in culture, suggesting a direct fibrogenic role for NKT paracrine signaling (118). In a co-culture system, NKT cells from the CD-HFD NASH model activate HSCs more strongly than CD8+ T cells, even though both immune populations mediate fibrogenic injury in vivo (121). Intriguingly, studies in CCl4 and DDC liver fibrosis models have reported that NKT cells can kill activated HSCs through expression of the NK receptor NKG2D, but its relevance to NASH has not been explored (122, 123).
Other Innate-Like T cells
Hepatic γδT cells are highly enriched in both healthy and diseased livers (124). Upon exposure to activated HSCs γδT cells induce apoptosis through the Fas-FasL pathway and depleting the liver of γδT cells in CCR6−/− mice accelerates MCD-NASH-related fibrosis (125). Like NKT cells, γδT cells appear to upregulate NK receptors to acquire HSC-targeting capacity under injury conditions (126). MAIT cells have recently emerged as a source of profibrogenic signaling in chronic liver diseases, but their contribution in NASH has not been clarified (127, 128).

Adaptive Immunity
Adaptive immune responses are potent inflammatory drivers in the progression to NASH. Hepatocyte and LSEC-derived signals recruit a broad repertoire of lymphocytes, resulting in the diffuse lobular infiltration that is a hallmark of NASH histology (116, 129). Global loss of adaptive immunity in Rag1−/− and B2M−/− mice is protective against steatosis, inflammation, and fibrosis in the choline-deficient high fat diet (CD-HFD) model, highlighting the overall contribution of adaptive immune cells to NASH disease (121).

Conventional T Cells
T cells may be especially important in promoting NASH and NASH-HCC (116, 130). The NASH T cell response is characterized by an enrichment for cytotoxic CD8+ T, TH1 differentiated CD4+ T and NKT cell populations (18, 121, 131–135). These immune shifts drive hepatocyte metabolic dysregulation through IFNγ, TNFα, and IL-17A signaling and production of the lymphotixin LIGHT (18, 121, 132, 133). Metabolic derangement in NASH also induces T cell pathology. Notably, CXCR6+ CD8+ T cells acquire hepatocyte killing activity in response to increased levels of the short-chain fatty acid acetate (136). Accordingly, depletion of T lymphocyte populations or disruption of their signaling activity is protective (115, 133, 137).

HSCs are an integral part of the sinusoidal niche and likely provide signals that influence T cell functions in NASH. Receptor-ligand analysis of their dense signaling networks includes potential chemokine interactions with T cells (19). Moreover, HSCs are the key hepatic source of vitamin A and its immunologically important metabolite RA (8). Vitamin A from HSCs is converted by neighboring LSECs to RA which primes CD4+ T cells to acquire a gut-homing phenotype mediated by α4β7 integrin and CCR9 expression (42, 138). These α4β7+ CD4+ T cells are key mediators of intestinal barrier disruption, increasing enterohepatic circulation of LPS and bacteria. Antagonism of this pathway in an experimental mouse model of NASH reduces hepatic inflammation and fibrosis (139).

T cells may act to restrain HSC fibrogenesis during NASH regression (Figure 2). In a HFD NASH model there was expansion of CD69+ CD103− CD8+ tissue resident memory T cells when mice were allowed to recover on a regular chow diet. These CD8+ memory T cells attract HSCs through the chemokine receptor CCR5 and induce HSC apoptosis via FasL. Adoptive transfer of regression-primed CD8+ memory T cells reduces the number of aHSCs and controls fibrosis (21).

B Cells
Intrahepatic B cell infiltration is associated with NASH progression (140, 141). Soluble markers related to B cell survival and activity including BAFF and IgG increase with worsening NASH severity, but the mechanisms by which B cells contribute to disease have not been characterized (142, 143). In HFD-NAFLD and MCD-NASH mouse models, B cells display prominent innate-like signaling function characterized by release of proinflammatory TNFα and other cytokines that activate HSCs and promote TH1 T cell activity (140, 144, 145). Future studies empowered by advances in scRNAseq and associated technologies may identify additional points of communication between B cells and HSCs (145). Potential interactions are hinted at, but not explored, in some of the first single cell analyses of NASH livers, including Cxcl12-Ccr4 in mouse and TNFRSF14-BTLA in human aHSCs and B cells, respectively (19, 146).

EMERGING AREAS
Cellular heterogeneity is now evident among HSCs and immune cell subsets as a result of increasing use of scRNAseq, raising new questions about how interactions between these cell types may be subdivided and targeted with greater precision (19, 20, 145, 147). At the same time, there is growing appreciation for antibiologic signaling by immune cells, especially during fibrosis regression. Future studies that can identify pro-fibrotic and antifibrotic cell types and interactions with greater precision will be of great value to the development of targeted therapies.

Single cell analysis of HSC populations in murine NASH recently identified a cluster of “inflammatory” HSCs characterized by reduced collagen scar production and increased immune and secretory pathway activity that may be more relevant to immune crosstalk (148). Separately, fibrogenic TREM2 CD90 “scar-associated” macrophages have been characterized in human cirrhosis (56). These may represent key pathologic cellular subsets worthy of therapeutic targeting. Likewise, depletion of other disease-associated subpopulations such as senescent HSCs, which are inflammatory, immune-stimulating in NASH, would be an appealing approach; however, these studies will rely on characterizing a unique molecular signature of HSCs to selectively target this cell type (149, 150). Any cell-directed therapies will need to avoid inhibition of beneficial activities by cells including “restorative” Ly-6Clo macrophages and miR-223-producing neutrophils. In support of this, additional work is needed to further define the cell subsets with antifibrotic roles, especially those that promote fibrosis regression.

CONCLUSIONS
Interactions between HSCs and hepatic immune cells clearly regulate fibrosis in NASH. HSCs collaborate with innate immune...
cell types to initiate hepatic inflammation in the transition from simple steatosis to steatohepatitis. They also undergo fibrogenic activation in response to inflammatory signaling, mediated by both innate and adaptive cell types, and signal back to those immune subsets, amplifying their activation (Figure 1) (3, 9). However, increasing evidence indicates that immune-HSC crosstalk is also tightly linked in the resolution of NASH injury and fibrosis regression. In specific contexts NK, NKT, and CD8+ T cells all induce HSC apoptosis to attenuate scar deposition (21, 108, 123). Neutrophil and macrophage subsets may blunt HSC activation and promote fibrosis regression as well (Figure 2) (69, 103).

Future studies need to establish the key signals that orchestrate the shift from pro- to anti-fibrotic signaling by immune cells. Many new candidate interactions are already suggested through single cell transcriptomic analyses, enabling a global assessment of immune cell interaction networks (147, 151, 152). Ultimately, functional validation will be necessary to establish which interactions are most impactful.

Careful dissection of timing and key regulators of HSC-immune interactions in NASH promises to clarify which therapeutic strategies will disrupt disease promoting pathways without interfering with the liver’s innate capacity for repair.

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JC and SF wrote the manuscript. Both authors contributed to the article and approved the submitted version.

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