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Hepatic stellate cells, liver innate immunity, and hepatitis C virus

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Abstract

Chronic hepatitis C virus (HCV) infection can cause liver damage, ranging from mild to more severe conditions, such as fibrosis and cirrhosis. Hepatic stellate cell (HSC) activation is a key event in HCV-induced liver fibrosis. HSCs express several HCV coreceptors that interact with HCV proteins, promoting liver fibrogenesis. In addition, HSCs have the ability to engulf apoptotic bodies of hepatocytes induced by HCV and trigger a profibrogenic response. Recent studies have suggested that HSCs may play a novel role in the liver innate immunity. HSCs enhanced differentiation and accumulation of regulatory T cells. HSCs-activated natural killer cells could produce γ-interferon that inhibits HCV replication. Importantly, HSCs possess functional Toll-like receptor-3 and retinoic acid-inducible gene I that can be activated by their ligands (poly I : C, 5′ppp-dsRNA), leading to the induction of interferon and inhibition of HCV replication in hepatocytes. These new observations highlight the importance of HSCs in liver immunity against HCV, which is the focus of this review paper.

Keywords

hepatic stellate cells; hepatitis C virus; innate immunity; retinoic acid-inducible gene I; toll-like receptor-3

Introduction

Because of the chronic nature of hepatitis C virus (HCV) infection and its high prevalence and significant morbidity of the resulting diseases, HCV is and will continue to be a serious global health threat for many years to come.[1] As a hepatitis virus, HCV infects human liver where the interactions between HCV and innate immunity play a key role in the immunopathogenesis of HCV disease. Unfortunately, the majority of HCV-infected subjects develop chronic infection that can result in liver fibrosis and cirrhosis. It is known that hepatic stellate cells (HSCs) are involved in HCV-induced liver fibrosis. HSCs are liver pericytes that reside in the space between parenchymal cells and sinusoidal endothelial cells of the liver.[2] HSCs are rich in vitamin A and store nearly 80% of retinoids of the whole body in its lipid droplets in the cytoplasm.[3,4] Interestingly, recent studies[5–15] suggest that HSCs participate in the liver immunity. In this paper, we review the recent development in HSC-mediated immunity and the significance of these new observations.

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Conflict of interest

The authors declare that there is no conflict of interest.

HSCs and HCV

HCV represents one of the major causes of liver fibrosis. The rate of progression of liver fibrosis varies widely in the chronic HCV infection, and progresses to cirrhosis within 20 years in an estimated 20–30% of individuals with chronic HCV infection.[16] The role of HSCs in HCV-mediated liver fibrosis has been well documented. HCV-infected hepatocytes release transforming growth factor-β1 (TGF-β1) and other profibrogenic factors that differentially modulate HSC expression of several key genes involved in liver fibrosis.[17] HCV infection-induced hepatocyte apoptosis is a common feature in chronic HCV infection.[18,19] Apoptosis results in the generation of apoptotic bodies (ABs), which are subsequently cleared by phagocytosis. Several studies showed that HSCs have the ability to engulf ABs through phagocytosis, which can trigger a profibrogenic response.[20,21] It was reported that ABs derived from HCV-infected Huh7 cells exhibited a more pronounced effect on profibrotic genes expression in HSCs than HCV-negative ABs.[22] Besides the indirect effects of HCV on HSCs function through infected hepatocytes, several studies[23–26] indicated that there is also a direct contact between HCV and HSCs. The potential interaction between HSCs and HCV is suggested by the observation that HSCs express high levels of CD81 protein,[23] a key entry coreceptor for HCV.[24] It has been demonstrated that the HCV E2 protein can directly bind to CD81 on HSC surface, inducing fibrogenic effects on HSCs.[25] In addition to HCV envelope protein, HCV core and nonstructural proteins have also been shown to affect HSC functions.[26] Recombinant HCV core and NS3 proteins could increase intracellular calcium concentration and reactive oxygen species production in activated HSCs.[26] HCV core protein could increase HSC proliferation, and NS3-NS5 protein preferentially induced pro-inflammatory cytokines in HSCs. The roles of HSCs in HCV infection-mediated liver fibrosis are summarized in Table 1.

HSCs and liver immunity

HSCs have recently been implicated to play a novel role in the liver immunity. It was reported that HSCs could induce vigorous natural killer T (NKT) cell responses *in vitro* and *in vivo*, and promote homeostatic proliferation of NKT cells.[13] In addition, HSCs could elicit antigen-specific T cells and inhibit bacterial infection in a Listeria monocytogenes infection model.[13] A recently study suggested that HSCs act as regulatory bystanders, enhancing differentiation and accumulation of regulatory T cells (Tregs), which may lie at the basis of the tolerogenic nature of the liver.[9] HSCs could function as antigen presenting cells, as they have the ability to process protein antigens and present peptides to $CD4^+$ and $CD8^+$ T cells.[13] Moreover, HSCs have been shown to express retinoic acid early inducible-1 (RAE1), cluster of differentiation 1d (CD1d), and major histocompatibility complex (MHC) I and II, and directly interact with immune cells, such as T cells,[13] NKT cells,[14] natural killer (NK) cells[10] (Table 2). HSCs also express several pattern recognition receptors, such as Toll-like receptors (TLRs)[12,30,31] and retinoic acid-inducible gene I (RIG-I),[8] indicating that HSCs possess innate immunity against pathogen infection.

HSCs and TLRs

The host innate immune system recognizes pathogens and responds to their stimuli mainly through TLRs. TLRs are key sensors of host innate immunity to pathogens. Several TLR members play a critical role in recognition of viral nucleic acids.[32] TLR-3 has a crucial role in virus-mediated innate immune responses,[33–35] as it recognizes dsRNA[36] that either constitutes the genome of one class of viruses or is generated during the life cycle of many viruses, including HCV.[33–35,37] Sensing through TLR-3 activates interferon (IFN) signaling pathway and induces the production of type I IFNs (IFN-α/β). IFN-α/β has been recognized as the first line of the TLR-3 activation-mediated antiviral response.[38] In addition, TLR-3

signaling also induces type III IFN expression.[39–41] Therefore, the activation of TLR-3 by its ligand poly I : C in viral target cells could inhibit virus infections, including HCV.[37] A very recent study[12] demonstrated that HSCs express functional TLR-3, activation of which induced production of IFN-β, and inhibited HCV replicon replication.[12] Our recent study[15] showed that TLR-3 signaling of HSCs could induce type III IFN expression, which contributed to HSCs-mediated HCV inhibition in hepatocytes. In addition to TLR-3, HSCs also express TLR-2,[29] TLR-4[30] and TLR-9.[31] HSCs express the stable levels of TLR-2 that respond to HCV core protein, inducing fibrogenic actions.[29] A recent study also showed that HCV core protein induces fibrogenic actions of HSCs via TLR-2 signaling pathway.[29] TLR-4 activation by lipopolysaccharides (LPS) in HSCs enhances TGF-β signaling and hepatic fibrosis.[30] HSCs express TLR-9 that are involved in liver fibrosis, as evidenced by TLR-9-deficient mice being resistant to liver fibrosis.[31]

HSCs and RIG-I

RIG-I is now well known as an important mediator of antiviral immunity. RIG-I can detect viral genomic RNA during negative-strand RNA virus infection[42] and trigger a type I IFN-mediated immune response that protects the host against viral infection.[43] RIG-I can recognize HCV genome, inducing innate immune response to restrict HCV replication in hepatocytes.[44] However, we know little about whether HSCs possess functional RIG-I signaling pathway and produce anti-HCV factors. Our recent studies examined whether HSCs have the ability to mount a RIG-I-mediated innate immunity that is effective in the control of HCV infection of human hepatocytes.[8] We demonstrated that HSCs (LX-2 cells) possess functional RIG-I that can be activated by the RIG-I ligand, resulting in the induction of IFNs and inhibition of HCV replication in hepatocytes.[8] This RIG-I signaling-mediated anti-HCV activity was potent, as when HCV JFH-1-infected hepatocytes were co-cultured with RIG-I-activated LX-2 cells or incubated in media conditioned with supernatant (SN) from RIG-I-activated LX-2 cells, HCV replication in hepatocytes was significantly suppressed.[8] Further investigation showed that RIG-I-activated LX-2 cells produced both type I IFN (IFN-β) and type III IFN (IFN-λ).[8] The role of IFNs in RIG-I-mediated HCV inhibition was evidenced by the observation that antibodies to type I IFN receptor or type III IFN receptor could compromise LX-2-SN-mediated anti-HCV effect in Huh7 cells.[8] The importance of RIG-I-activated IFN signaling pathway in LX-2 cell-mediated anti-HCV activity was further demonstrated in the experiments, showing that inhibition of RIG-I by specific siRNA could block the IFN induction by 5′ppp-dsRNA.[8] These new observations provide additional evidence to support the notion that the activation of RIG-I signaling in HSCs can help with the control of HCV infection/replication in the liver.

HSCs and NK cells

In normal liver, HSCs are in a quiescent state and represent 5–8% of the total number of liver cells.[4] HSCs become activated following liver injury, and activated HSCs enhanced migration and deposition of extracellular matrix components, resulting in liver fibrosis.[27,28] Recent studies demonstrated that activated HSCs could induce NK cell activation, resulting in IFN-γ production that has the ability to inhibit HCV replication.[5,6] Conversely, NK cells had the ability to kill activated HSCs, and subsequently inhibit liver fibrosis in both mice [11] and humans.[6,7] In mouse models of liver fibrosis, NK cells could ameliorate liver fibrosis via killing of activated HSCs in a RAE-1/NKG2D-dependent and tumor necrosis factor-related apoptosis-inducing ligand-dependent manner.[11] Furthermore, the NK cells-mediated anti-fibrogenic effects are suppressed during advanced liver injury, which is likely due to increased production of TGF-β and expression of suppressor of cytokine signaling 1 in intermediately activated HSCs.[5] It was reported that NK cells from HCV-infected patients are more efficient in inducing apoptosis of activated HSCs than NK cells from healthy

subjects, suggesting that the interactions between HSCs and NK cells has a crucial role in chronic HCV infection-related liver disease.[7]

Conclusion

Although great progress has been made in the research field of HSCs and liver fibrosis, limited information is available about the role of HSCs in liver immunity. As shown in Table 2, recent studies[5–15] by several groups have clearly shown that HSCs are involved in the regulation of liver immunity. It was shown that HSCs could act as a regulatory bystander, enhancing differentiation and accumulation of Tregs.[9] Activated HSCs can also induce NK cell activation, which results in suppression of liver fibrosis and HCV infection.[5–7,11] Furthermore, TLR-3 or RIG-I-activated HSCs could produce both type I and type III IFNs that could inhibit HCV replication in hepatocytes.[8,12,15] These novel observations, although require further *ex vivo* and *in vivo* studies to confirm, highlight the importance of HSCs in liver immunity against HCV infection.

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Table 1

HSCs in HCV-mediated liver fibrosis

HCV and its proteins	HSCs	References
HCV	Induces liver injury, which activates HSCs	27,28
	Engulfs ABs through phagocytosis triggers a profibrogenic response in HSCs	20,21
E2	Upregulates matrix aetalloproteinase-2 expression, increasing degradation of the normal hepatic extracellular matrix in HSCs	25
Core	Induces fibrogenic actions and stimulates intracellular signaling pathway in HSCs	26,29
NS3-NS5	Induces pro-inflammatory cytokines in HSCs	26

HCV, hepatitis C virus; HSC, hepatic stellate cell.

Table 2

HSCs in liver innate immunity

HSCs	Liver innate immunity	References
Regulatory bystanders	Enhancing differentiation and accumulation of regulatory T cells	9
Interaction with immune cells	Express retinoic acid early inducible-1, CD1d, and MHC I and II, directly interact NK cells, NKT cells and T cells	10,13,14
NK cell activation	Inhibit liver fibrosis and hepatitis C virus replication	5–7,11
Inhibition of bacterial infection	Against bacterial infection in a Listeria monocytogenes infection model	13
Inhibition of viral infection	Express functional Toll-like receptors and retinoic acid inducible gene I receptors, activation of which induce the production of type I and type III IFNs	8,12,15

HSC, hepatic stellate cell; NK, natural killer.