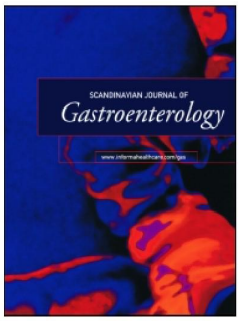

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REVIEW ARTICLE

Immune regulation in chronic hepatitis C virus infection

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ABSTRACT

The immunological result of infection with Hepatitis C virus (HCV) depends on the delicate balance between a vigorous immune response that may clear the infection, but with a risk of unspecific inflammation and, or a less inflammatory response that leads to chronic infection. In general, exhaustion and impairment of cytotoxic function of HCV-specific T cells and NK cells are found in patients with chronic HCV infection. In contrast, an increase in immune regulatory functions is found primarily in form of increased IL-10 production possibly due to increased level and function of anti-inflammatory Tregs. Thus, the major immune players during chronic HCV infection are characterized by a decrease of cytotoxic function and increase of inhibitory functions. This may be an approach to diminish intrahepatic and systemic inflammation. Finally, there has been increasing awareness of regulatory functions of epigenetic changes in chronic HCV infection. A vast amount of studies have revealed the complexity of immune regulation in chronic HCV infection, but the interplay between immune regulation in virus and host remains incompletely understood. This review provides an overview of regulatory functions of HCV-specific T cells, NK cells, Tregs, IL-10, and TGF-β, as well as epigenetic changes in the setting of chronic HCV infection.

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Introduction

Infection with Hepatitis C virus (HCV) is a major health problem, and estimated number of individuals with chronic HCV infection (CHCV) is 130–150 million worldwide. Infection with HCV leads to chronic infection in 60–80% of infected individuals. Among individuals with CHCV infection 20–30% eventually develop liver fibrosis and ultimately cirrhosis.[1] It remains incompletely understood why some individuals are able to spontaneous clear the infection, while others develop CHCV. Likewise, it is largely unknown why some individuals progress to fibrosis and cirrhosis while others do not. A strong immune response is crucial for spontaneous clearing of the virus. In contrast, a strong immune response during CHCV may result in increased inflammation and possibly increased risk of developing fibrosis and cirrhosis. Thus, timing and regulation of the host immune system is essential having a major effect on outcome in CHCV.

The immune response upon HCV infection is complex, and several mechanisms have been suggested to be involved. Among the contributors to immune responses against HCV is HCV-specific CD8 + T cells considered to be of great importance. In addition, Natural Killer (NK) cells have an important function in the very early response against HCV infection, but besides this well described cytotoxic function, NK cells may also have a function in immune regulation. Furthermore, regulatory T cells (Tregs), Interleukin (IL)-10, and Transforming growth factor (TGF)-β have all been suggested to be involved in the regulation of the immune responses against HCV infection. Finally, microRNAs (miRNAs) have recently been shown to be regulators of the immune response during the acute as well as the chronic course of HCV infection.

The aim of this review is to describe immune regulation in CHCV. The focus will be on immune regulation that favors clearance of acute HCV infection versus establishment of CHCV as well as on the role of immune regulation in development of liver inflammation and progression to fibrosis.

T cells in chronic HCV infection

The main effector cell in the adaptive immune response directed against HCV is the HCV-specific CD8 + T cell. HCV-specific CD8 + T cells inhibit viral replication through a non-cytolytic and a cytolytic pathway, and can be determined by direct staining of T cells with HCV-specific tetramers binding to T cell receptors specific for the HCV peptide.[2] Non-cytolytic effector functions are primarily mediated by secretion of Interferon (IFN)-γ while cytolytic effector functions involve perforin-granzymes and Fas ligand (CD95L).[2] During acute HCV infection an effective HCV-specific CD8 + T cell response has the capacity to spontaneous clearing of the virus and to establish long-lived memory T cells. Although, this does not provide permanent protective immunity a reduced risk of developing CHCV upon re-exposure is observed.[3] The detailed mechanisms of the optimal HCV-specific CD8 + T cell response are incompletely understood, but studies in chimpanzees have demonstrated the importance of both

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CD8 + and CD4 + memory T cells, and that inhibition of HCV replication by HCV-specific CD8 + T cells is based on both the cytolytic cell-cell contact and the non-cytolytic IFN-γ production.[4–6] Thus, the optimal CD8 + T cell response is directed against multiple epitopes in the HCV protein. However, HCV-specific CD4 + T cells are required for the establishment of a sufficient response by virus specific CD8 + T cells [2] (Figure 1). This is exemplified in cases with low CD4 + T cell counts such as Human Immunodeficiency Virus (HIV) infection resulting in increased risk of CHCV and accelerated development of fibrosis.[7–9]

However, in the majority of recently infected individuals, the HCV-specific CD8 + T cells fail to clear the virus resulting in CHCV. It is evident that in CHCV-infected individuals HCV-specific CD8 + T cells are impaired in function although characterization of HCV-specific CD8 + T cells is challenged due to a frequency of only 0–5.5% of intrahepatic CD8 + T cells while the frequency of HCV-specific CD8 + T cells in peripheral blood is below 0.5%.[10,11] Interestingly, it has recently been shown that one-third of HCV-specific CD8 + T cells in CHCV were characterized by a naïve phenotype indicating that the CD8 + T cells have yet not met their cognate antigen despite on-going viral replication.[12] This may be a result of viral escape mechanism and/or functionally impairment of antigen-presenting cells, or exhausted HCV-specific CD8 + T cells. The term 'exhaustion' has been used to describe functionally

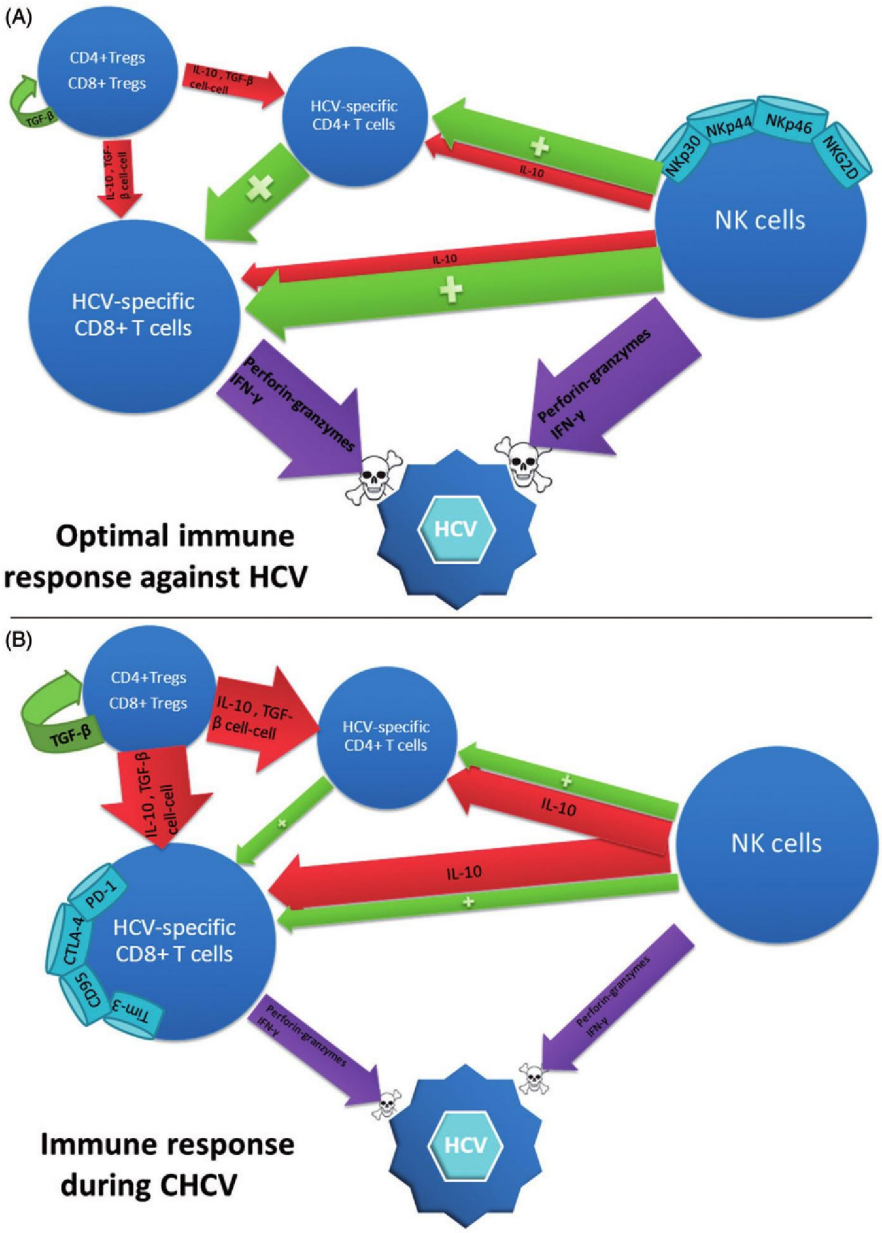

Figure 1. An optimal immune response against HCV (A) is characterized by active cytotoxic HCV-specific CD8 + T cells and NK cells. Furthermore, NK cells have increased expression of activating receptors (NKp30, NKp44, NKp46, and NKG2D) and stimulate both HCV-specific CD4 + and CD8 + T cells. Likewise, HCV-specific CD4 + T cells stimulate HCV-specific CD8 + T cells. Finally, the inhibition of Tregs is low. In contrast, the immune response during chronic HCV infection (B) is characterized by impaired cytotoxic function of HCV-specific CD8 + T cells and NK cells, and increased regulatory function in NK cells (IL-10 production). Likewise, HCV-specific CD4 + T cells are impaired, and the inhibition by Tregs through cell-to-cell contact, IL-10, and TGF-β is increased. Finally, apoptosis markers and inhibitory receptors (PD-1, CD95, CTLA-4, and TIM-3) are increased on HCV-specific CD8 + T cells.

and proliferative impairment as well as expression of inhibitory molecules on T cells.[13] In CHCV infection, several studies have demonstrated that proliferation of and cytokine production [IL-2, IFN-γ, and Tumor Necrosis Factor (TNF)-α] by HCV-specific CD8 + T cells is reduced in peripheral blood as well as intrahepatically compared to both non-HCV-specific CD8 + T cells in the same individual and to proliferation and cytokine production in individuals that managed to clear the infection.[14,15] Furthermore, HCV-specific CD8 + T cells have been shown to display increased expression of both programmed cell death (PD-1) and CD95 compared to non-HCV-specific CD8 + T cells suggesting HCV-specific CD8 + T cells to be more prone to undergo apoptosis.[14,16] Several other inhibitory receptors such as CTLA-4 and Tim-3 have been demonstrated to be overexpressed on HCV-specific CD8 + T cells compared to non-HCV-specific CD8 + T cells in the same CHCV-infected individual [17,18] (Figure 1).

Importantly, in CHCV only HCV-specific CD8 + T cells seem to be exhausted while non-HCV-specific CD8 + T cells have normal function.[14] Furthermore, the exhaustion of the intrahepatic HCV-specific CD8 + T cells seem to be more severe compared to peripheral HCV-specific CD8 + T cell.[16] Several mechanisms may be involved in exhaustion including continuous antigen stimulation,[19] increased stimulation by inhibitory cytokines (IL-10 and TGF-β) (see below), or simultaneous impairment of HCV-specific CD4 + T cells and antigen-presenting cells (reviewed in Ref. [13]).

It is still uncertain whether HCV-specific CD8 + T cells contribute to or confine intrahepatic inflammation during CHCV. However, due to the specific impairment or exhaustion of HCV-specific CD8 + T cells, other mechanisms have been suggested to be the main driver of intrahepatic inflammation during CHCV including non-HCV-specific T cells and monocytes.[20]

NK cells in chronic HCV infection

Natural Killer (NK) cells have a main function in the innate immune response against many viral infections.[21] Their function is mediated through release of granzyme- and perforin-containing granules.[21] Furthermore, NK cells are a source of early IFN-γ production during viral infections. IFN-γ limits viral replication, activates macrophages, and promotes a Th1 response.[21] In addition, an indirect regulative function of NK cells through IL-10 production affecting T cells and dendritic cells (DC) has been described.[22] NK cells are defined phenotypically as CD3-CD16 + CD56+, and constitute 30–60% of lymphocytes in the liver and 5–15% of peripheral blood lymphocytes.[23,24] NK cells have been suggested to play a role in the spontaneous clearance of HCV, since a strong association between spontaneous clearance and homozygosity for the NK cell receptor gene KIR2DL3 and its ligand HLA-C1 group alleles has been found.[25] This is supported by a study that showed that the capability of KIR2DL3 + NK cells to clear HCV infection was mediated in the innate phase of infection prior to seroconversion.[26] Furthermore, an early multifunctional NK cell response including increased IFN-γ production and increased expression of activating receptors (NKp30, NKp44, NKp46, and NKG2D) have been found in individuals clearing HCV compared to individuals developing CHCV.[27,28] Finally, in individuals exposed to HCV an increased NK cell response was followed by an increase in IFN-γ production by T cells in individuals spontaneous clearing HCV compared to individuals developing CHCV.[27,29] This supports a role for NK cells in the crosstalk between innate and adaptive immunity, and emphasizes the role of NK cells in spontaneous clearing of HCV infection (Figure 1).

In CHCV-infected individuals, the peripheral NK cells are reduced in number, and polarized towards an impaired IFN-γ production, altered phenotype, and increased cytolytic activity compared to healthy individuals.[24,30–33] The impairment in number and IFN-γ production are comparable to the described impairment seen in HCV-specific T cells during CHCV.[24,34,35] In addition, intrahepatic NK cells display altered function and phenotype compared to NK cells in peripheral blood, and also intrahepatic NK cells in CHCV-infected individuals have reduced IFN-γ production and increased IL-10 production compared to healthy controls.[36,37]

Intrahepatic NK cells more frequently express the two activation molecules, NKp46 and the TNF-related apoptosis-inducing ligand (TRAIL), compared to peripheral NK cells. [30,38,39] TRAIL and NKp46 have shown to be involved in lysis of activated CD4 + T cells and activated hepatic stellate cells suggesting them to be involved in inhibition of inflammation and progression of fibrosis.[40–43] In contrast, one study demonstrated a positive association between the intrahepatic proportion of NKp46 + NK cells and intrahepatic inflammation score.[39] Thus, the decreased antiviral IFN-γ production of NK cells in CHCV-infected individuals seems to be supplemented by increased cytolytic activity towards activated hepatic stellate cells possibly protecting against fibrosis (Figure 1).

Regulatory T cells in chronic HCV infection

Essential in immune regulation are Tregs with important functions in the delicate balance between an adequate protective immune response and immunopathology. Best-defined are CD4 + CD25 + natural Tregs. Natural Tregs are produced either in the thymus or they can be produced by peripheral proliferation. In contrast, adaptive Tregs (Th3 and Tr1 cells) exclusively develop in the periphery when mature T cells are activated (reviewed in Ref. [44]). Adaptive Tregs are poorly defined and their function therefore less well understood. In this review, Tregs refers to natural Tregs.

Chronic HCV infection (CHCV) has been associated with an unbeneficial Treg function suggestive to contribute to failure of spontaneous clearing of HCV. Indeed, higher proportion of Tregs in HCV-infected individuals compared to healthy controls is well-documented both in the peripheral blood and intrahepatic, and Tregs may be the direct cause of CD4 + T cell unresponsiveness during CHCV.[45–50] Also, Tregs have been suggested to be positively associated with HCV RNA [51] and the degree of inflammation.[52] However, one study

found equal relative proportion of Tregs in HCV-infected individuals with both mild and severe fibrosis.[50]

Lately, it has been shown that HCV core-protein upregulate Forkhead box P3 (FoxP3), IL-10, and triggers expansion of Tregs.[53,54] Also, *in vitro* studies have demonstrated HCV-infected hepatocytes to induce Tregs through the production of TGF-β.[55] Furthermore, pre-therapeutic HCV-specific TGF-producing T cells predict lack of clearance in HIV-HCV co-infected individuals supporting the hypothesis of Tregs suppressing sufficient immune responses during CHCV [56] (Figure 1). Altogether, these studies suggest that Tregs are involved in the CHCV pathogenesis. It is tempting to assume that suppression of the Treg response could be beneficial in the setting of CHCV. However, a recent phase-2a placebo controlled trial with oral anti-CD3 immunotherapy, which is considered biologically active in the gut through induction of regulatory T cells, showed a decrease in viral load and liver enzymes along with an increase in Tregs suggesting Tregs to be beneficial.[57] This may well be explained by the heterogeneity of Tregs complicating the understanding of the role of Tregs in immune responses in general (earlier reviewed in Refs. [58] and [59]). Furthermore, the role of Tregs in relation to HCV is probably a two-edged sword with a harmful suppression of immune responses directed against HCV leading to lack of clearing of the virus, and on the other hand, a beneficial suppression of the unspecific immune responses thereby limiting the harmful inflammation.

The mechanism whereby Tregs suppress effector T cells in CHCV is unclear, but includes cell-to cell communication and/or IL-10 signaling.[60] Thus, HCV is able to induce antigen-specific Tregs to suppress antiviral T cell responses in an antigen-specific manner.[61] Interestingly, a recent study demonstrated an inverse association between proportion of CD4+ T cell and their expression of PD-1, CLTA-4, and FoxP3, suggesting a homeostatic mechanism controlling the immune response during CHCV [62] (Figure 1). Treg proliferation during CHCV is inhibited by PD-1, modulated by CD8+regulatory T-cells secreting IL-10. Subsequently, inhibition of HCV-specific cytotoxic CD8+T-cells leads to lack of eradication of virus-infected cells resulting in lack of control of the infection [63] (Figure 1). Furthermore, increased numbers of intrahepatic CD8+Tregs have been found in chronic HCV-infected individuals,[64] but the opposite has been shown as well.[49] Interestingly, IL-10 secreting CD8+Tregs present in specific areas of the liver may prevent liver fibrosis.[64] Indeed, further studies are warranted in order to determine the influence and distribution of these cells in patients with CHCV.

Growing evidence support the idea of DCs being involved in initiating and sustaining efficient T cell responses. HCV-DC were shown to be poor activators of CD4+T cells, but this could be reversed by the elimination of CD4+CD25+cells [65] suggesting Tregs to be critical in lack of clearance of the virus. Overall, the interaction between Tregs and dendritic cells may be of importance in HCV pathogenesis (reviewed in Ref. [66]).

Finally, HCV promotes Treg differentiation via TGF-β signaling.[55] This suggests a link between HCV-induced TGF-β signaling, Treg differentiation and suppression of the effective host immunity against HCV which ultimately leads to chronicity as shall be discussed below.

IL-10 in chronic HCV infection

Interleukin (IL)-10 is a cytokine produced by several cells in the immune system including Tregs, but the main source of IL-10 is macrophages. IL-10 inhibits major histocompatibility complex (MHC) class II on monocytes and macrophages by limiting the production of pro-inflammatory cytokines and chemokines which ultimately leads to impaired pathogenic control and/or reduced immunopathology.[67,68] IL-10 signaling is believed to play a role in the lack of viral eradication, especially in the early phases of infection and subsequent progression to CHCV.[69] Thus, high IL-10 levels in the acute phase of an infection has been shown to suppress HCV-specific effector CD4+and CD8+T-cells, eventually leading to CHCV.[70,71] Thus, the addition of IL-10 to HCV primed CD8+T-cells impaired the expansion of these cells, and the effect of IL-10 suppression diminished over time.[69] This suggests that IL-10 secretion plays an important role in the suppression of the specific immunological response in the early, rather than in the late phases, during the primary infection. Both IL-10 secreting CD4+T cells and IL-10 secreting CD8+T cells have been documented to play a part in CHCV pathogenesis. As an anti-inflammatory cytokine IL-10 may also prevent progression of fibrosis in individuals with CHCV, and an association between high levels of IL-10 and lower levels of fibrosis has been found.[72] Furthermore, treatments with a recombinant IL-10 agonist have been shown to decrease development of fibrosis in patients with CHCV and non-response after antiviral treatment.[73] Thus, although evidence is sparse IL-10 does seem to protect against fibrosis. Further studies are required in order to understand the mechanism and to determine the long term effects of IL-10 on fibrosis.

TGF-β in chronic HCV infection

Transforming growth factor (TGF)-β is a cytokine produced by a wide range of different cell types. All non-parenchymal cells in the liver including Kupffer cells produce TGF-β. Hepatocytes have been reported to store and release TGF-β, but whether they produce it themselves remains unclear.[74] Furthermore, TGF-β levels in the liver have been shown to be upregulated directly by HCV core proteins.[75] In parallel with IL-10 TGF-β is also believed to play a role in suppressing a sufficient immune response against HCV infection. TGF-β inhibits Th1 and Th2 cell expansion and promotes Treg dominated T-cell responses.[74] Inhibition of Th1 and Th2 expansion, in turn, is believed to prevent an effective response against HCV infection, thereby driving the infection towards chronicity. TGF-β has a direct suppressing effect on Th1 and Th2 differentiation and an indirect suppression on Th1 and Th2 response via Tregs.[74] TGF-β plays a vital role on the differentiation of Tregs, induces FoxP3 expression in Tregs, and

prevents apoptosis by promoting anti-apoptotic pathways and blocking activation of pro-apoptotic pathways.[76]

Excess TGF-β activity has been suggested to contribute to development of liver fibrosis and subsequently cirrhosis.[77] Thus, hepatic fibrosis develops due to an injury to the cells in the liver. Especially long-term injury such as that caused by chronic viral hepatitis, autoimmune responses, or alcohol promotes fibrosis formation.[78] Myofibroblasts, originating from the hepatic stellate cell (HSC),[79] promote formation of the extracellular matrix (ECM), and TGF-β plays a vital role in the activation of HSC, linking TGF- β expression and fibrosis formation in the liver.[80] Furthermore, higher level of TGF-β in serum and liver in HCV-infected individuals and a strong correlation between fibrosis formation and TGF-β levels in general has been found.[81] However, one study found that TGF-β was not associated with fibrotic stage in HCV-infected individuals.[82] In conclusion, further studies are needed in order to clarify TGF-β's part in HCV derived progression of fibrosis and chronic disease.

MiRNA in chronic HCV infection

MicroRNAs (MiRNAs) are small non-coding RNAs that post-transcriptionally regulate expression of target genes via degradation of messenger (m)RNA transcripts or inhibition of mRNA translation.[83] MiRNAs function in the complex balanced regulation of immune activation, inflammation, and host–virus interactions (reviewed in Refs. [84–87]). From the intracellular compartment, miRNAs can be secreted to the blood stream as stable nuclease-resistant entities.[88] Extracellular stability and the fact that miRNAs are aberrantly expressed during the course of many diseases, including viral hepatitis and cancer, make them promising biomarkers. In addition, increasing evidence points to extracellular miRNAs as carriers of biological functions such as cell to cell signaling.[89,90] Cross-talk between miRNA and HCV is an emerging research area, and as discussed in the following it has been established that several miRNAs have important functions in immune regulation and intrahepatic inflammation during CHVC.

The most frequent miRNA in the liver, miR-122, accounts for approximately 70% of all miRNA in normal liver tissue, and plays a central role in hepatocyte homeostasis and regulation of fatty acid and cholesterol metabolism.[91–94] In mouse models, genetic deletion of miR-122 results in microsteatosis, inflammation, fibrosis, and carcinogenesis in the liver.[93,94] An interesting function of miR-122 is the effect on HCV replication as miR-122 binds directly within the HCV genome and promotes viral translation and replication.[95–97] Other miRNAs including miR-448, miR-196/196a, and miR-199a inhibit HCV replication via direct binding to the HCV genome.[98–101]

MiRNA and immune regulation in HCV infection

In addition to a direct role in HCV replication, miR-122 is also thought to have important functions in hepatic immune regulation and during the course of CHCV.[102] First, several studies indicate that serum miR-122 is a stable biomarker of inflammation and liver injury in CHCV and is associated with aspartate transaminase (AST) and alanine transaminase levels (ALT).[103–107] Second, it has been suggested that regulation of hepatic inflammation by miR-122 is mediated by CCL2, a small cytokine expressed in inflammatory cells and hepatocytes that recruits monocytes, memory T cells and DC.[94] CCL2 and the CCL2-receptor was found to be upregulated in miR-122 deficient mice leading to increased infiltration of inflammatory cells and higher levels of the pro-inflammatory cytokines IL-6 and TNF-α.[94] Thus, the overall immune regulatory function of miR-122 in the liver seems to be downregulation of inflammation. This illustrates the complex functions of miRNAs in virus–host interactions, since the impact of a single miRNA can be diverse and affect several biological systems. Other miRNAs, miRNA-107 and miRNA-449a, may regulate the CCL2-pathway in CHCV by upregulated CCL2 expression.[108]

A unique regulator of the immune system is miR-155, because it is implicated in multiple immunological processes such as regulation of lymphocyte homeostasis, initiation, and regulation of chronic inflammation and regulation of immunologic memory (reviewed in Ref. [109]). In CHCV, miR-155 has been proposed to promote inflammation because miR-155 induced production of TNF-α in monocytes from CHCV treatment-naïve individuals.[106] Increased levels of miR-155 have been detected both in liver tissue, serum, and monocytes from HCV-infected individuals.[106,110] In monocytes from HCV-infected individuals, a variety of HCV-ligands such as HCV core, NS3, and NS5 proteins and toll-like receptors (TLR)4 and TLR8 ligands may initiate upregulation of miR-155 and increased TNF-α production.[106] In another study, HCV RNA replication was positively correlated with expression of miR-155.[111] Importantly, it was demonstrated that upregulation of miR-155 in hepatocytes promoted cell proliferation and inhibition of apoptosis while miR-155 inhibition induced arrest of cell differentiation. This suggests miR-155 not only to be a regulator of liver inflammation and hepatocyte proliferation but also as a possible initiator of HCC.[110]

Increased TNF-α production induced by miR-155 has been shown both in hepatic macrophages from HCV-infected individuals and in alcohol-treated Kupffer cells in a mouse model of alcoholic liver disease,[106,112] indicating that upregulated miR-155 could be a common feature for several liver-specific diseases. HCV may regulate TLR-signaling via miRNAs. Viral components are recognized by TLRs that initiate an intracellular activation cascade leading to production of type I IFN, e.g., IFN-α/IFN-β, and pro-inflammatory cytokines. A suggested immune evading feature of CHCV is downregulation of TLR-7 and TLR-3 in peripheral blood leading to decreased IFN-α production, decreased control of the infection and increased risk of CHCV.[113,114] Several studies have demonstrated that miR-758 may be a regulator of these TLRs since HCV infection led to increased miR-758 with a subsequent decrease in TLR3/TLR7 expression and IFN-α/IFN-β production in peripheral blood and HCV-infected liver cells.[113,115] However, no correlations were found between TLR3 and TLR7 and viral load or histopathological staging and grading of the liver tissue.[113] In addition, miRNA-21 has been proposed as a

regulator of TLR signaling since HCV infection of human hepatocytes led to downregulation of IFN-α production and enhanced viral replication via the TLR signaling pathway.[116] Expression of miR-21 in serum from HCV-infected individuals correlated with both viral load, fibrosis, and serum liver transaminase levels.[117] As with miR-155, a role as driver of HCC has also been proposed for miR-21.[118–121] Other miRNAs with proposed functions in CHCV are summarized in Figure 2.

MiRNA and liver fibrosis in chronic HCV infection

The role of miRNA in HCV-induced liver fibrosis has been extensively reviewed elsewhere,[122] and the main priority here is to give an overview of the most studied miRNAs implicated in development of liver fibrosis. Liver fibrosis is a complicated process, and it is thought that miRNA is involved at several levels (Figure 2). A possible two-phase response in the course of CHCV has been demonstrated for miR-122. During the early stages of CHCV and fibrosis, miR-122 is increased and positively associated with necrosis and inflammation measured by serum ALT/AST levels.[103,105,107,123,124] In the advanced stages, miR-122 is reduced both in the circulation and in hepatic tissue and correlates with fibrosis stage.[123–125] In contrast, miR-29 has anti-fibrotic effects and inhibits the synthesis of fibrillary collagen [126,127]. In hepatocytes from individuals with CHCV and fibrosis, miR-29 is significantly downregulated and collagen synthesis is enhanced.[128,129] Finally, in fibrotic HCV-infected individuals, miR-21 is correlated with fibrotic stage. This is explained by a pro-fibrinogetic mechanism where miR-21 targets SMAD7, an inhibitor of fibrosis.[130]

MiRNA and treatment in chronic HCV infection

Recent evidence suggests that miRNA may influence treatment outcome of HCV infection. Thus, several miRNAs were differential expressed in HCV-infected individuals with sustained virologic response (SVR) compared to non-responders after standard treatment.[131] For example, miR-122 has been investigated as a marker of treatment outcome in CHCV. Thus, one study demonstrated that non-responders had significant lower hepatic miR-122 levels compared to responders.[132] Another study showed that serum levels of miR-122 remained low in individuals with SVR but increased to baseline in non-responders or individuals relapsing after therapy, suggesting that serum miR-122 may function as a marker of treatment outcome after standard treatment.[133] However, miR-122 has not been investigated in relation to direct-acting antivirals (DAAs). Recently, a possible therapeutic role of antisense oligonucleotide targeting miR-122 (miravirsen) has been found, as a phase IIa human clinical trial demonstrated promising results with efficiently reduced HCV RNA in individuals with CHCV infection.[134] Finally, hepatic miR-155 expression has been shown to be upregulated in CHCV, and

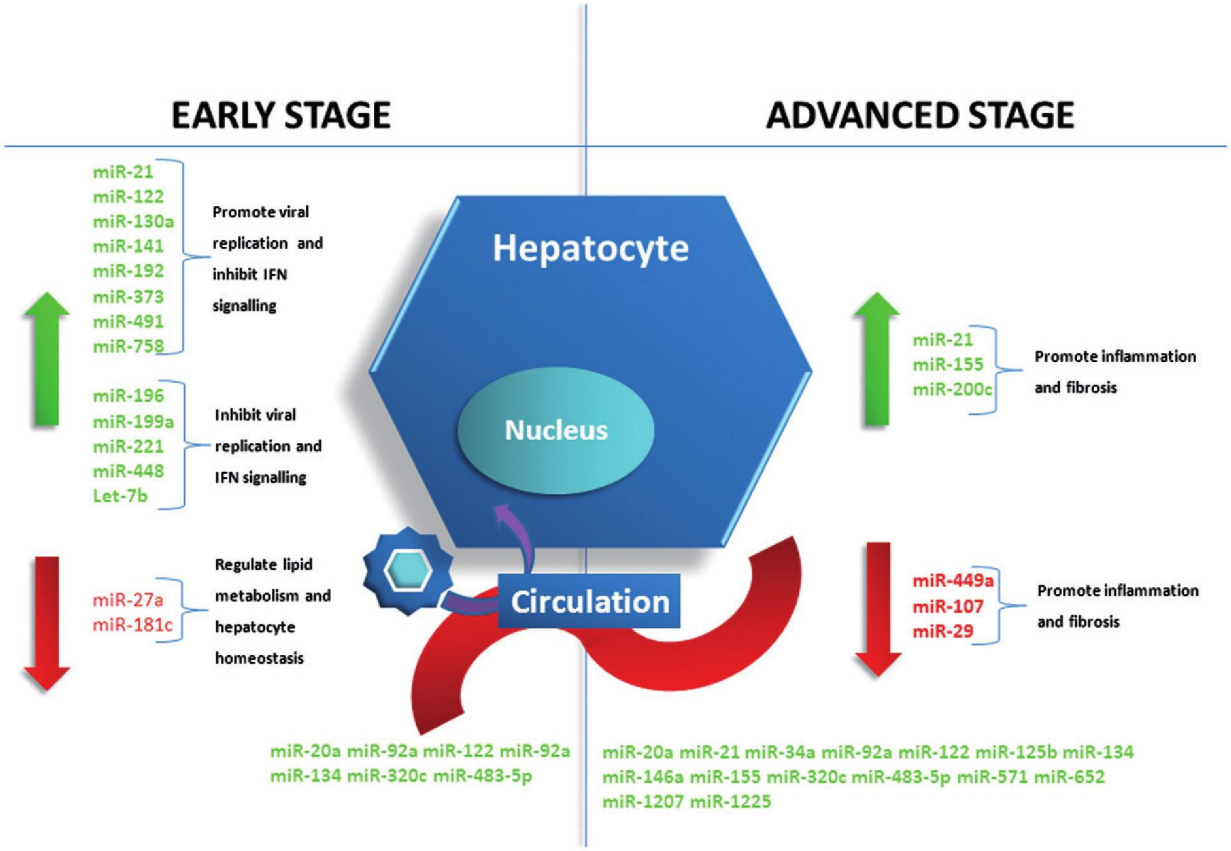

Figure 2. Overview of differentially expressed miRNAs in hepatocytes and in the circulation in the course of HCV infection. Several miRNAs have important functions in viral replication in early stages of infection and promotion of inflammation and fibrosis in advanced stages of infection. Upregulated miRNAs are marked with green while downregulated miRNAs are marked with red.

elevated serum levels were associated with lower viral load and SVR to standard treatment.[135] This highlights the complexity of many miRNAs and immune regulation in general, as upregulated miRNAs and immune activation may limit HCV replication, but lead to subsequent tissue damage in CHCV.

Antiviral treatment and immune functions

The standard care of treatment for CHCV has until recently been pegylated IFN-α and ribavirin, but interferon-free treatment with DAAs has become available, and increase in SVR rates from 40–50% to above 90% for genotype 1 has been demonstrated.[136] However, IFN-α is still a major part of the recommendation on treatment of CHCV,[137] and the impact of IFN-α on the immune system remains of interest, as studies have demonstrated lack of full recovery of immune function after SVR. Thus, interferon-treatment was not able to counteract the exhaustion of HCV-specific T cells.[138,139] However, the SVR rate is higher in individuals treated with IFN and ribavirin during acute HCV infection compared to individuals with chronic infection, and early IFN-treatment has been reported to rescue both proportion and function of HCV-specific CD8 + T cells.[138,140] Furthermore, a recent clinical trial showed lower Treg frequency in those patients with complete early virological response than those without,[141] emphasizing the importance of a multifunctional and vigorous T cell response for clearing of the virus. Interestingly, a recent study demonstrated that IFN-free regimens restored the proliferative capacity of CD8 + T cells in contrast to IFN-based treatment, suggesting that IFN may be involved in the irreversible CD8 + T cell exhaustion despite SVR.[142] This is in line with our own results showing little difference comparing the effect of IFN-containing treatment and IFN-free treatment on T cell homeostasis and CD127 expression after SVR.[143] Similar conditions seem to be apparent in the NK cell compartment. Thus, recent studies showed a rapid decrease of NK cell activation and normalization of NK cell function towards the level in healthy individuals after IFN-free regimens [144,145] in contrast to IFN-based treatment which stimulated the activation and cytotoxic function of NK cell but reduced the IFN-γ production.[146,147]

Accordingly, the effect of IFN on the immune system is massive and seems in some aspects to be irreversible while new IFN-free regimen seems to have little or negligible effect on the immune system and level of inflammation.

Conclusions

The natural course of HCV infection is intriguing in the form of the great disparity between individuals. Some individuals may more or less asymptomatically spontaneous clear the infection, while other develops chronic infection and subsequently fibrosis, cirrhosis, hepatocellular carcinoma, and eventually death. The host immune response and the regulation of this play a major role in the course of HCV infection. However, the immune responses and the immune regulation in CHCV are not completely understood. A strong immune response in chronic HCV infection may restrain the virus, but may also result in inflammatory damages in the liver leading to fibrosis and cirrhosis. Thus, regulating the immune response may be beneficial in chronic HCV infection. This is illustrated by an exhaustion and cytotoxic functionally impairment of HCV-specific CD8 + T cells and NK cells, as well as increased level of Tregs, and epigenetic changes.

The increased immune regulatory functions and establishment of low grade inflammation in CHCV may be comparable to the changes seen in infection with HIV during combined antiretroviral treatment. Although, the increased immune regulatory functions and level of inflammation in HIV infection may be of a greater degree. However, these areas have been extensively investigated in HIV infection compared to CHCV, and further studies investigating immune regulation in CHCV is warranted.

The therapy of CHCV is in a great development and the continuous development and improvement of DAAs is promising for treating CHCV. However, still it remains of interest to develop the understanding of the pathogenesis of HCV, as the infection often has a covert course and may be revealed many years after infection with inflammation, fibrosis, and further complications at risk. A better understanding of the immune responses during CHCV contributes to even more efficient therapy and hopes for vaccine development for the millions of individuals with chronic HCV infection.

Disclosure statement

The authors have no conflict of interest to disclose.

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