TOPIC HIGHLIGHT

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Role of macrophages and monocytes in hepatitis C virus infections

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Abstract

A number of studies conducted over many years have shown that hepatitis C virus (HCV) can infect a variety of cell types. *In vivo* infection of monocytes, macrophages, and dendritic cells by HCV has been frequently shown by a number of researchers. These studies have demonstrated replication of HCV by detecting the presence of both negative genomic strands and a variety of non-structural HCV proteins in infected cells. In addition, analyses of genome sequences have also shown that different cell types can harbor different HCV variants. Investigators have also done preliminary studies of which cellular genes are affected by HCV infection, but there have not yet been a sufficient number of these studies to understand the effects of infection on these cells. Analyses of *in vitro* HCV replication have shown that monocytes, macrophages and dendritic cells can be infected by HCV from patient sera or plasma. These studies suggest that entry and cellular locations may vary between different cell types. Some studies suggest that macrophages may preferentially allow HCV genotype 1 to replicate, but macrophages do not appear to select particular hypervariable regions. Overall, these studies agree with a model where monocytes and macrophages act as an amplification system, in which these cells are infected and show few cytopathic effects, but continuously produce HCV. This allows them to produce virus over an extended time and allows its spread to other cell types.

Core tip: We review the evidence of infection of monocytes and macrophages by hepatitis C virus (HCV), both *in vivo* and *in vitro*. There are two innovative ideas: (1) the hypervariable region of HCV may vary due to host range of variants in addition to immune pressure; and (2) a novel model of the role of macrophages and monocytes in HCV infections is proposed that is consistent with current evidence.

INTRODUCTION

Hepatitis C virus (HCV) is a cause of liver diseases as well as other extrahepatic pathologies. A combination of the drugs ribavirin and pegylated interferon is usually used as therapy. More recently other drugs have been approved that may increase the chances of successful treatment. These other drugs are designed to interfere with specific viral proteins. It is important to understand how HCV replicates so that rational therapies can be designed.

This review concentrates on the infection of monocytes, macrophages, and dendritic cells by HCV so that we can better understand the life cycle of HCV.

Macrophages are phagocytic cells that are found in all tissues. They are produced from the differentiation of monocytes. Macrophages are part of the innate immune system, but they also have a role in adaptive immunity by stimulating immune cells to respond to pathogens. Liver macrophages are called Kupffer cells, which comprise as much as 25% of the cells in the liver[1,2], while brain macrophages are called microglial cells, which comprise about 10% of the cells in the brain[3]. There is evidence that Kupffer cells are involved in lipid homeostasis in the liver[4], and that impaired activation of Kupffer cells can result in hepatic steatosis and insulin resistance[5]. Similarly, microglial cell disfunction in the central nervous system (CNS) can result in neurodegenerative diseases[3].

HCV has not only been implicated in liver diseases, but also in a number of extrahepatic pathologies, including mixed cryoglobulinemia, B-cell non-Hodgkin lymphoma, and porphyria cutanea tarda[6]. Most of these diseases are thought to be due to indirect effects of HCV infection, such as chronic inflammation, autoantibody production, and the deposition of antibody-antigen complexes. A number of HCV-related nervous system disorders have also been found, including neurological problems such as confusion or seizures, cognitive problems such as fatigue and depression, and neuropathies such as pain and sensory problems[7]. There is some evidence of direct involvement of HCV in some of these disorders, but others are also probably due to secondary effects of the infection. Since there are a large number of extrahepatic problems in HCV-infected individuals, studies have been done to determine the types of cells that can be infected by HCV. Many different cell types and tissues have been reported to be infected by HCV[8], but proof that they are the cause of particular problems in patients is mostly lacking. In this review, we will be concentrating on the evidence of infection of monocytes and macrophages, and the possible consequences of infection of these cells.

HCV is a positive-strand virus about 9.6 kb in size. Replication is through a negative-strand intermediate. Evidence of negative strands of HCV in cells is often used as indicating that the HCV present in particular cells is replicating. When HCV replicates, it produces a number of well characterized non-structural proteins not found in the virus: NS2, NS3, NS4A, NS4B, NS5A, and NS5B. The presence of these proteins in cells is interpreted as resulting from HCV replication. The proteins in the virion are core, the envelope proteins E1 and E2, and the ion channel protein p7. There is also one protein produced from a ribosomal frameshift during translation, called F or AFRP. All of these proteins are made from cleavage of a polyprotein of approximately 3000 amino acids. The HCV genome contains a 341 untranslated nucleotide segment at the 5' end of the genome called the 5'UTR or 5'NTR that is important for replication[9]

and translation[10,11]. The region of the genome located at the amino end of the E2 protein is called the hypervariable region 1 (HVR1). The 5'UTR is the most conserved region of the genome and the HVR1 is the most variable.

IN VIVO EXPERIMENTS SHOWING INFECTION OF MONOCYTES

Soon after assays were developed for HCV, reports that the replicative form, the negative strand of the HCV genome, could be detected in peripheral blood mononuclear cells (PBMC)[12-15]. However, it was later shown that false positives of HCV RNA could be a problem. Therefore, modifications to the PCR protocols were instituted, including the use of rTth polymerase. Since then, over 20 additional studies have shown the presence of negative strands of HCV RNA in PBMC using modified PCR methods[8]. In addition, many studies have shown that B-cells, T-cells and other cell types *in vivo* also can have detectable negative strands of HCV RNA.

After PBMC were shown to be infected by HCV, studies were performed to determine which cell types were infected. Flow cytometry was used to separate monocytes from other cell types of PBMC from HCV-infected patients. These cells appeared to contain the Core protein of HCV[14], suggesting that HCV was inside the cells. *In situ* hybridization to locate negative strand HCV RNA has also been used to show that HCV infects monocytes/macrophages, B cells, and possibly T-cells[16]. Immunofluorescence experiments also have shown a variety of HCV non-structural proteins in the cells, confirming they were infected[16]. RT-PCR has also been used to test for negative strands of HCV. PBMC separated into different cell types by immunomagnetic selection of cell types show both HCV positive and negative strands of HCV RNA in granulocytes, monocytes, and B-cells, but not in T-cells or thrombocytes[17]. Other studies using immunomagnetic selection of cell types have shown negative HCV strands in monocytes/macrophages, T-cells, and B-cells[18] and in B-cells, monocytes, and polymorphonuclear leukocytes, but not in T-cells[19]. These studies all agree that, in some patients, negative strands can be found in monocytes or macrophages. HCV has also been found to bind to natural killer, monocytes, and B-cells found in PBMC, suggesting a role for these cells as carriers of HCV to various tissues[20].

Fluorescence microscopy and immunohistochemistry have also been used to show that HCV in monocytes is present and replicating. For example, studies have shown the presence of NS4/NS4B[16,21-23] and NS5/NS5A[24,25] in monocytes or macrophages.

As studies have shown that different cell types in PBMC harbor replicating HCV, other work investigated differences in quasispecies in these tissues. The predominant hypervariable region 1 (HVR-1) sequence of HCV has been shown to be different in liver, PBMC, and plasma[26] or liver, PBMC, and serum in patients[27,28]. However, these studies did not look at particular cell types in these

samples. Significantly different sequences have been found in different cell types found in PBMC, including monocytes and B-cells[29]. Different types of monocytic cell lineages have been investigated, with CD14+, CD16++ and CD14++, CD16++ but not CD14+, CD16- cells being found infected[30]. These studies suggest that different cell types allow replication of slightly different versions of HCV.

Studies have also investigated liver tissue for infection by HCV. One early study used *in situ* hybridization to locate HCV and found a few positive cells, which were most likely lymphocytes or macrophages[31], but they did not positively identify the cell types. A more recent study stained cells for both HCV Core and NS4 as well as cell type specific markers CD68, CD3, and CD20[21]. They found that macrophages were the major target of HCV in liver, heart, kidney, and bone marrow cells. Other *in situ* hybridization and immunohistochemical studies of liver cells have not stained for both HCV and the cell type; more studies are therefore needed.

Since cognitive impairment is a manifestation of HCV infection, a number of studies of HCV infection of neuronal cells have been performed. HCV negative strands were found in brain tissues from three patients that were obtained from autopsies[32]. They also showed that in two patients the serum and brain derived HCV had different HCV genotypes. As CD14 was detected in the brain tissue samples but not CD2 (T-cells) or CD19 (B-cells), they suggest that macrophages/monocytes were infected. HCV sequences from brain, liver, lymph node, and serum samples were also found to have differences in the HVR1 and IRES regions, providing evidence of tissue compartmentalization[33]. They also investigated translation efficiency of the IRES, and found the quasispecies in the brain have lower translation efficiency than that of liver or serum. RT-PCR has been used to show that HCV is found in postmortem brain tissue[34]. Immunostaining was also performed to determine that microglial/macrophage cells (CD68+ and CD45+) were infected by HCV, while neurons and oligodendroglial cells did not stain. Brain macrophages/microglial cells have also analyzed for cytokine and chemokine expression[35]. Higher levels of expression of the cytokines interleukin 1α and tumor necrosis factor α, interleukin 1β, interleukin 12, and interleukin 18 and the chemokines interleukin 8, interleukin 16, and interferon-inducible protein 10 were found in HCV infected CD68+ brain cells. They suggested that infected leukocytes could pass HCV to cells in the CNS.

Two groups have investigated expression of toll-like receptors (TLR) in monocytes of infected individuals. One study found that TLRs 2, 5, 6, 7, 8, 9, and 10 mRNA levels were all upregulated compared to uninfected individuals[36]. In addition, MD-2, CD14, and MyD88 were also upregulated in monocytes. However, they did not check the monocytes to see if they were infected with HCV. A second study looked primarily at expression levels in PBMC[37]. They found TLRs 4, 7, and 8 upregulated in CD14+ monocytes, as well as TNFα, IL-6, and IL-12p35 in PBMC. Therefore, TLR7 and TLR8, which are activated by single-strand RNA, were found upregulated by both groups, and TLR3 did not significantly change in either study, but they disagreed on the other TLR. This could be due to a number of factors, including patient differences and how the monocytes were collected. One other group found no significant differences in the levels of CD14+CD16- and CD16+CD14- monocytes after HCV infection[38], although there were differences in response to TLR8-ligation or LPS stimulation. Therefore, they suggest that CD16+CD14- monocytes do not appear to have a role in HCV pathogenesis, although they may differentiate into Kupffer or Dendritic cells. However, there is no reason to expect that levels of monocytes would be related to the pathogenesis of HCV, so their conclusions don't seem reasonable.

Dendritic cells (DC), like macrophages, are derived from monocytes. RT-PCR has been used to show that monocyte-derived DCs contain HCV RNA[39]. Circulating DCs have also been shown to be positive for HCV RNA[40,41]. Analysis of the HVR-1 quasispecies suggests that HCV in dendritic cells were different than serum. Myeloid dendritic cells (mDC) separated from plasmacytoid dendritic cells (pDC), and then sequenced, had HVR-1 that was often significantly different between the DC and sera. HCV was detected in monocytes, but not in mature DC produced from the monocytes after stimulation with granulocyte macrophage colony-stimulating factor (GM-CSF) and IL-4[42]. They found the metabolism of the DCs to be altered compared to monocytes from uninfected patients. It may be that their conversion of monocytes to DC only works well on HCV-negative cells, *e.g.*, HCV-infection may inhibit the conversion. However, a recent review of the effects of chronic HCV infection on dendritic cells found some agreement that there are lowered numbers of mDC and pDC in infected individuals, but there have not been any convincing functional defects found in the cells[43].

These *in vivo* studies show that monocytes, macrophages, and dendritic cells can be infected by HCV. The HCV sequence results suggest that different cell types can select slightly different HCV variants, producing separate pools or compartments. This suggests that at least part of the reason that the major HCV variants change in patients over extended times is due to changes in cell tropism. A recent study of one HCV patient over an extended time suggests that the proportions of particular variants changes over time[44]. It is hard to reconcile their observations with the hypothesis that immune pressure is the driving force for genetic variation of HCV. Instead, a combination of changes in cell tropism and the immune system adaptive system trying to eliminate the virus better accounts for HCV variation over extended times. Studies that have investigated monocyte/macrophage cell responses to HCV have not been extensive enough to reach firm conclusions about changes in gene expression by HCV infected cells.

IN VITRO EXPERIMENTS INVESTIGATING REPLICATION OF HCV IN MONOCYTES

As there is significant evidence for infection of monocytes, macrophages, and dendritic cells *in vivo*, a number of groups have studied infection of these cell types *in vitro*. The first study of monocytes/macrophages *in vitro* isolated the cells from healthy donors[45]. They then incubated them with HCV positive sera. There was a low level of infection, and the HVR-1 sequences of the serum and cultured samples were very similar. A second study testing the infection of Kupffer cells found no significant infection of them[46].

Monocytes/macrophages isolated from PBMC of healthy donors were shown to be infectable by sera from HCV-infected patients[47]. They found negative strands of HCV present in the cells, and the quasispecies of the 5'UTR and NS5B regions were investigated by single-strand conformation polymorphism (SSCP) and sequencing. They found some sequence changes in either the NS5B or 5'UTR for samples from some patients. Monocytes/macrophages already infected with HIV have been found to be more infectable by HCV, and they had higher levels of HCV RNA than cells that were not pre-infected with HIV[48]. PBMC from uninfected individuals that was incubated with HCV genotype 4, and then separated into cell types by flow cytometry, has detectable negative strands by RT-PCR and immunofluorescence staining against core and E1 24 h post infection[49]. The staining was most frequently in monocytes, as determined by flow cytometry.

A monocytoid cell line, THP-1, has been tested for susceptibility to infection by HCV[50]. The presence of HCV envelope protein E2 was found inside cells using immunofluorescence, and HCV RNA was detectable in cell culture supernatants seven days after infection. However, recently, another group found HCV to enter THP-1 cells in a manner that was insensitive to anti-CD81 blocking antibodies, but the HCV did not replicate[51]. These cells, therefore, may not be useful as an *in vitro* model for studying HCV replication. However, they showed that Kupffer cells in the liver produce low but detectable levels of interferon-β in HCV-infected individuals and THP-1 cells also produce interferon-β in response to HCV infection, suggesting that THP-1 cells were responding similarly to Kupffer cells. Livers of HCV-infected individuals were also analyzed for the source of IL-1β production. Staining of liver sections using antibodies against both IL-1β and CD68 showed that IL-1β was produced in intrahepatic macrophages[52]. *In vitro* cultured THP-1 cells also produced IL-1β in response to cell culture-derived HCV (HCVcc). The THP-1 cells had peak IL-1β production 3 h after virus exposure. The virus was found to enter THP-1 cells in a method independent of the CD81 co-receptor. UV-inactivated virus also caused the IL-1β response, suggesting that viral replication was not necessary for the response.

Dendritic cells have been tested *in vitro* for susceptibility to HCV infection[53]. Negative strands were detected in both immature and mature DC within 2 d after infection. Quasispecies analysis of the HVR-1 and SSCP of the 5'UTR showed minor differences between HCV in the patient sera and the DC HCV. One study of the infection of isolated dendritic cells, both myeloid DC and plasmacytoid DC, using HCVcc provided evidence that HCV uses different methods of entry into each of the cell types[54]. They also provide evidence that the HCV localizes in different places in the two cell types: endosomes in plasmacytoid DC and in lysosomes in myeloid DC. The virus thus likely uses different sets of receptors to enter each cell.

A culture system that has been extensively studied uses primary macrophages generated from umbilical cord blood[55]. In this system, HCV from serum or plasma of an HCV-infected individual was used to infect the primary macrophages (Figure 1). After about 7 d, the HCV in the cell culture supernatant was used to infect other cell types, including B-cells, T-cells, and macrophage-like neuronal cells (Table 1). Analysis of the 5'UTR of HCV genotype 1 showed that there were only minor differences between the serum/plasma and the HCV produced by the other cell types[56]. However, variants in serum that contained a large deletion did not replicate in the macrophages[57]. This suggests that the macrophages are replicating most but not all HCV variants. Additional support for this was seen when HCV genotype 3 was analyzed[58]. The 5'UTR for the HCV genotype 3 cultured *in vitro* had changed significantly, and was now similar to HCV genotype 1. This suggested that the macrophages are selecting sequences similar to HCV genotype 1. Culturing in other cell types did cause selection of particular HVR-1. It was also found that culturing in macrophages produced the same variants as seen in one patient's serum almost 18 mo later, suggesting macrophages tend to produce the same variants *in vitro* as seen *in vivo*. Overall, these results suggest that macrophages in HCV genotype 1 individuals are producing HCV that is a little more infectious than that found in the serum.

These results suggest that studying the infection of monocytes and macrophages *in vitro* and *in vivo* may yield insights into the life cycle of the virus. What is the role of monocytes and macrophages? Are they important for sustaining the infection or just innocent bystanders and only transiently infected?

MODEL OF THE ROLE OF MACROPHAGES

Most HCV investigators have likely regarded the productive infection of macrophages by HCV as either something that does not happen or of little importance. Researchers who have studied the *in vivo* infection of macrophages and monocytes, however, have provided a substantial amount of evidence that they are infected, as detailed above. There has been little agreement of whether infection of monocytes or macrophages is im-
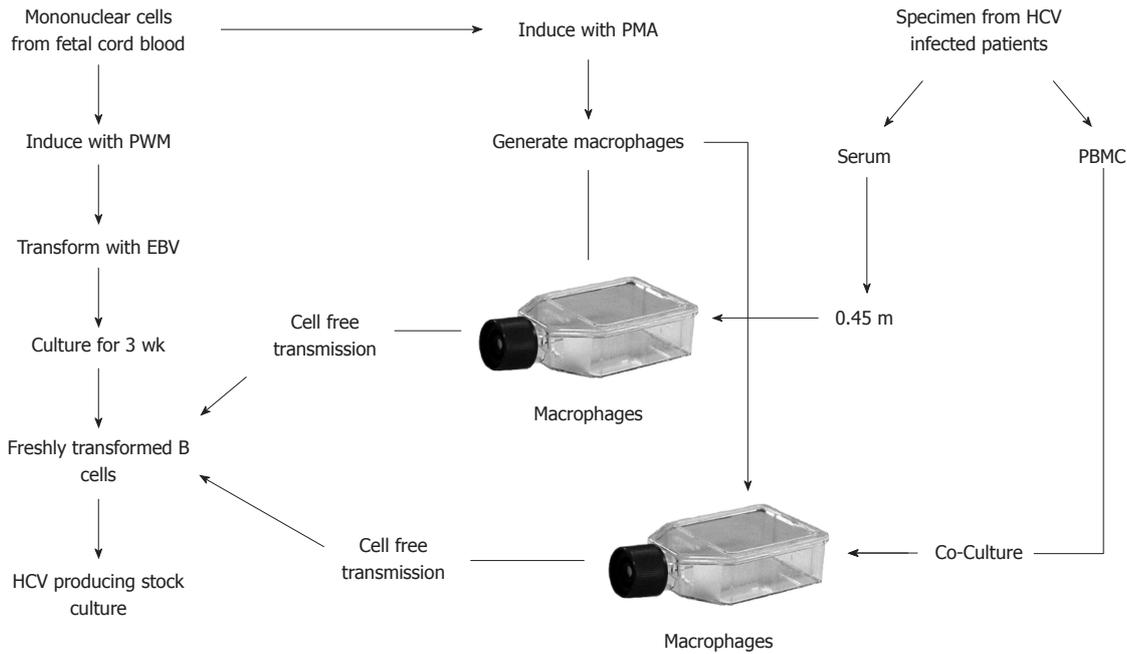
Figure 1 Isolation method for studying *in vitro* replication of hepatitis C virus. Macrophages are generated from mononuclear cells in fetal cord blood and infected by hepatitis C virus (HCV). Adapted from reference[55]. EBV: Epstein-Barr virus; PWM: Pokeweed mitogen; PMA: Phorbol-12-myristate-13-acetate; PBMC: Peripheral blood mononuclear cells.

Table 1 Transmission of hepatitis C virus into various hematopoetic and liver cells

	Short term replication	Long term replication
T-cells[1]	+	-
B-cells[2]	+	+
Monocytes/macrophages[3]	+	-
Neuronal precursors[4]	+	+
Liver cells[5]		
Kupffer cells	+	-
Hepatocyte	+	+/-

Adapted from reference[55]. [1]T-cells isolated from human fetal cord blood; [2]B-cells immortalized by infection with transforming Epstein-Barr virus; [3]Monocyte/macrophages: adherent cells stimulated with phorbol-12-myristate-13-acetate; [4]Recently isolated neuronal cells from fetal human brain; [5]Freshly isolated liver cells from liver biopsies. Kupffer cells are liver macrophages and hepatocytes are liver endothelial cells.

portant for the long term consequences of HCV infection. Some work has been performed on the infection of neuronal cells *in vivo*, and it has been suggested that infection of microglial/macrophage cells may allow HCV to pass through the blood-brain barrier to infect brain cells such as astroglial cells[34,35,48]. This has been described as a "Trojan horse" mechanism.

Evidence that HCV RNA purified from various tissues (compartments) has small differences in their HVR and/or 5'UTR sequences suggests that different versions of HCV replicate in different tissues. In addition, *in vitro* evidence suggests that different cell types also preferentially replicate slightly different versions of HCV. Of the cell types investigated *in vitro*, monocytes/macrophages appear to have sequences that are closest to those found in the sera of patients. This suggests that monocytes/macrophages have an important role in HCV replication *in vivo*. The Trojan horse mechanism that has been proposed for infection of cells in the brain may also be valid for other tissues. Therefore, monocytes/macrophages in HCV infection may act as a carrier of the infection to other cells. The monocytes/macrophages get infected, but do not themselves have significant cytotoxic effects or metabolic problems when infected. This allows them to be infected for longer periods of time. Instead, they replicate HCV in a relatively non-specific manner for as long as they survive. Macrophages select against defective HCV, probably, in other regions of the HCV genome. Macrophages in HIV-1 infections have been shown to be "holding compartments" for HIV-1, where membranous labyrinths form and contain the virus[59]. Macrophages may also act in a similar manner with HCV and therefore produce and/or hold a lot of virus. Overall, we propose a model where HCV infects macrophages. The macrophages replicate the HCV and then pass it on to other cell types. Other cell types, on the other hand, often show effects of HCV infection, including cytopathic effects such as cell death. It may be that these cell types, when infected, produce the diseases we associate with long term infection by HCV, such as liver damage due to the cytopathic effects of HCV on hepatocytes. It should be noted that there are many types of macrophages, so each particular type may act slightly differently with regards to HCV, but our model extrapolates from the ones that have been studied to date.

What are the clinical implications of the proposed role of macrophages/monocytes? First, macrophages

are fairly long-lived cells, and generally survive for weeks. It has been proposed that the presence of CD14 on the cells supports survival of the cells[60]. This long life, even after HCV infection, suggests that not only is HCV produced for extended periods, but the cells can also produce cytokines that affect other immune cells such as B-cells and T-cells[60,61]. The cytokines can cause cytopathic effects on other nearby cells. Therefore, the infection of long-lived cells may partially explain the long treatment times needed to eradicate HCV. Second, results of *in vitro* studies may explain differences in therapy for different HCV genotypes. As described above, HCV genotype 1 readily infects macrophages *in vitro*, but HCV genotype 3 does not. Since therapy for HCV genotype 1 needs to be done for longer periods than HCV genotype 3[60], this may be due in part to the different cell tropisms for the two genotypes. Evidence in support of this has been shown in one report that showed that in patients that did not have a sustained virologic response to combination therapy with ribavirin and pegylated interferon-α, 88% were infected with genotypes 1 or 4, while 24% were infected with genotypes 2 or 3[62]. Some patients were infected with two genotypes. Third, studies of extrahepatic diseases resulting from HCV infection suggest the presence of replicating virus in peripheral blood or bone marrow-derived lymphocytes in patients with mixed cryoglobulinemia[63], in carotid plaques[64], and in peripheral blood leukocytes of patients with lichen planus[65]. The researchers did not determine which specific cell types contained replicating HCV in any of these reports, but it is likely that monocytes are infected. Unfortunately, we can find no studies that have investigated which specific cell types produce replicating HCV in any studies of extrahepatic diseases caused by HCV infection. Last, one group has shown CD68-positive macrophages in liver, heart, kidney, and bone marrow in autopsy specimens from patients that had hepatocellular carcinoma and cardiomyopathies[21]. This study therefore suggests that many different tissues can contain infected macrophages that may cause disease, either by passing HCV or indirectly by releasing signaling molecules.

The study of HCV replication to date has mostly concentrated on the JFH1/HuH 7.5 model system, to the neglect of studying HCV isolated from patients. This system is not relevant for the studies of the infection of a variety of cell types by the natural virus, which will be needed to let us understand how HCV causes disease. Although the work performed to date has advanced our understanding of HCV, the field needs to compare the results obtained from the model systems with studies using the natural virus to obtain a better understanding of how HCV is able to replicate in infected individuals.

REFERENCES

1   **Kita H**, Mackay IR, Van De Water J, Gershwin ME. The lymphoid liver: considerations on pathways to autoimmune injury. *Gastroenterology* 2001; **120**: 1485-1501 [PMID: 11313320 DOI: 10.1053/gast.2001.22441]

2   **Dong Z**, Wei H, Sun R, Tian Z. The roles of innate immune cells in liver injury and regeneration. *Cell Mol Immunol* 2007; **4**: 241-252 [PMID: 17764614]

3   **Aguzzi A**, Barres BA, Bennett ML. Microglia: scapegoat, saboteur, or something else? *Science* 2013; **339**: 156-161 [PMID: 23307732 DOI: 10.1126/science.1227901]

4   **Baffy G**. Kupffer cells in non-alcoholic fatty liver disease: the emerging view. *J Hepatol* 2009; **51**: 212-223 [PMID: 19447517 DOI: 10.1016/j.jhep.2009.03.008]

5   **Stienstra R**, Saudale F, Duval C, Keshtkar S, Groener JE, van Rooijen N, Staels B, Kersten S, Müller M. Kupffer cells promote hepatic steatosis via interleukin-1beta-dependent suppression of peroxisome proliferator-activated receptor alpha activity. *Hepatology* 2010; **51**: 511-522 [PMID: 20054868 DOI: 10.1002/hep.23337]

6   **Ko HM**, Hernandez-Prera JC, Zhu H, Dikman SH, Sidhu HK, Ward SC, Thung SN. Morphologic features of extrahepatic manifestations of hepatitis C virus infection. *Clin Dev Immunol* 2012; **2012**: 740138 [PMID: 22919404 DOI: 10.1155/2012/740138]

7   **Monaco S**, Ferrari S, Gajofatto A, Zanusso G, Mariotto S. HCV-related nervous system disorders. *Clin Dev Immunol* 2012; **2012**: 236148 [PMID: 22899946 DOI: 10.1155/2012/236148]

8   **Revie D**, Salahuddin SZ. Human cell types important for hepatitis C virus replication in vivo and in vitro: old assertions and current evidence. *Virol J* 2011; **8**: 346 [PMID: 21745397 DOI: 10.1186/1743-422x-8-346]

9   **Friebe P**, Lohmann V, Krieger N, Bartenschlager R. Sequences in the 5' nontranslated region of hepatitis C virus required for RNA replication. *J Virol* 2001; **75**: 12047-12057 [PMID: 11711595 DOI: 10.1128/JVI.75.24.12047-12057.2001]

10   **Rijnbrand RC**, Lemon SM. Internal ribosome entry site-mediated translation in hepatitis C virus replication. *Curr Top Microbiol Immunol* 2000; **242**: 85-116 [PMID: 10592657 DOI: 10.1007/978-3-642-59605-6_5]

11   **Wang C**, Sarnow P, Siddiqui A. Translation of human hepatitis C virus RNA in cultured cells is mediated by an internal ribosome-binding mechanism. *J Virol* 1993; **67**: 3338-3344 [PMID: 8388503]

12   **Wang JT**, Sheu JC, Lin JT, Wang TH, Chen DS. Detection of replicative form of hepatitis C virus RNA in peripheral blood mononuclear cells. *J Infect Dis* 1992; **166**: 1167-1169 [PMID: 1328405 DOI: 10.1093/infdis/166.5.1167]

13   **Qian C**, Camps J, Maluenda MD, Civeira MP, Prieto J. Replication of hepatitis C virus in peripheral blood mononuclear cells. Effect of alpha-interferon therapy. *J Hepatol* 1992; **16**: 380-383 [PMID: 1336787 DOI: 10.1016/S0168-8278(05)80674-9]

14   **Bouffard P**, Hayashi PH, Acevedo R, Levy N, Zeldis JB. Hepatitis C virus is detected in a monocyte/macrophage subpopulation of peripheral blood mononuclear cells of infected patients. *J Infect Dis* 1992; **166**: 1276-1280 [PMID: 1385547 DOI: 10.1093/infdis/166.6.1276]

15   **Zignego AL**, Macchia D, Monti M, Thiers V, Mazzetti M, Foschi M, Maggi E, Romagnani S, Gentilini P, Bréchot C. Infection of peripheral mononuclear blood cells by hepatitis C virus. *J Hepatol* 1992; **15**: 382-386 [PMID: 1332999 DOI: 10.1016/0168-8278(92)90073-X]

16   **Sansonno D**, Iacobelli AR, Cornacchiulo V, Iodice G, Dammacco F. Detection of hepatitis C virus (HCV) proteins by immunofluorescence and HCV RNA genomic sequences by non-isotopic in situ hybridization in bone marrow and peripheral blood mononuclear cells of chronically HCV-infected patients. *Clin Exp Immunol* 1996; **103**: 414-421 [PMID: 8608640 DOI: 10.1111/j.1365-2249.1996.tb08296.x]

17   **Lerat H**, Rumin S, Habersetzer F, Berby F, Trabaud MA, Trépo C, Inchauspé G. In vivo tropism of hepatitis C virus genomic sequences in hematopoietic cells: influence of viral load, viral genotype, and cell phenotype. *Blood* 1998; **91**: 3841-3849 [PMID: 9573022]

18   **Laskus T**, Radkowski M, Piasek A, Nowicki M, Horban A,

Cianciara J, Rakela J. Hepatitis C virus in lymphoid cells of patients coinfected with human immunodeficiency virus type 1: evidence of active replication in monocytes/macrophages and lymphocytes. *J Infect Dis* 2000; **181**: 442-448 [PMID: 10669324 DOI: 10.1086/315283]

19 **Crovatto M**, Pozzato G, Zorat F, Pussini E, Nascimben F, Baracetti S, Grando MG, Mazzaro C, Reitano M, Modolo ML, Martelli P, Spada A, Santini G. Peripheral blood neutrophils from hepatitis C virus-infected patients are replication sites of the virus. *Haematologica* 2000; **85**: 356-361 [PMID: 10756359]

20 **Natarajan V**, Kottilil S, Hazen A, Adelsberger J, Murphy AA, Polis MA, Kovacs JA. HCV in peripheral blood mononuclear cells are predominantly carried on the surface of cells in HIV/HCV co-infected individuals. *J Med Virol* 2010; **82**: 2032-2037 [PMID: 20981790 DOI: 10.1002/jmv.21906]

21 **Matsumori A**, Shimada M, Obata T. Leukocytes are the major target of hepatitis C virus infection: Possible mechanism of multiorgan involvement including the heart. *CVD Prev Control* 2010; **5**: 51-58 [DOI: 10.1016/j.cvdpc.2010.04.005]

22 **Sansonno D**, Cornacchiulo V, Racanelli V, Dammacco F. In situ simultaneous detection of hepatitis C virus RNA and hepatitis C virus-related antigens in hepatocellular carcinoma. *Cancer* 1997; **80**: 22-33 [PMID: 9210705]

23 **Benkoël L**, Biagini P, Dodero F, De Lamballerie X, De Micco P, Chamlian A. Immunohistochemical detection of C-100 hepatitis C virus antigen in formaldehyde-fixed paraffin-embedded liver tissue. Correlation with serum, tissue and in situ RT-PCR results. *Eur J Histochem* 2004; **48**: 185-190 [PMID: 15208088]

24 **Pham TN**, King D, Macparland SA, McGrath JS, Reddy SB, Bursey FR, Michalak TI. Hepatitis C virus replicates in the same immune cell subsets in chronic hepatitis C and occult infection. *Gastroenterology* 2008; **134**: 812-822 [PMID: 18243182 DOI: 10.1053/j.gastro.2007.12.011]

25 **Sansonno D**, Gesualdo L, Manno C, Schena FP, Dammacco F. Hepatitis C virus-related proteins in kidney tissue from hepatitis C virus-infected patients with cryoglobulinemic membranoproliferative glomerulonephritis. *Hepatology* 1997; **25**: 1237-1244 [PMID: 9141444 DOI: 10.1002/hep.510250529]

26 **Maggi F**, Fornai C, Morrica A, Vatteroni ML, Giorgi M, Marchi S, Ciccorossi P, Bendinelli M, Pistello M. Divergent evolution of hepatitis C virus in liver and peripheral blood mononuclear cells of infected patients. *J Med Virol* 1999; **57**: 57-63 [PMID: 9890422]

27 **Okuda M**, Hino K, Korenaga M, Yamaguchi Y, Katoh Y, Okita K. Differences in hypervariable region 1 quasispecies of hepatitis C virus in human serum, peripheral blood mononuclear cells, and liver. *Hepatology* 1999; **29**: 217-222 [PMID: 9862869 DOI: 10.1002/hep.510290117]

28 **Navas S**, Martín J, Quiroga JA, Castillo I, Carreño V. Genetic diversity and tissue compartmentalization of the hepatitis C virus genome in blood mononuclear cells, liver, and serum from chronic hepatitis C patients. *J Virol* 1998; **72**: 1640-1646 [PMID: 9445070]

29 **Ducoulombier D**, Roque-Afonso AM, Di Liberto G, Penin F, Kara R, Richard Y, Dussaix E, Féray C. Frequent compartmentalization of hepatitis C virus variants in circulating B cells and monocytes. *Hepatology* 2004; **39**: 817-825 [PMID: 14999702 DOI: 10.1002/hep.20087]

30 **Coquillard G**, Patterson BK. Determination of hepatitis C virus-infected, monocyte lineage reservoirs in individuals with or without HIV coinfection. *J Infect Dis* 2009; **200**: 947-954 [PMID: 19678757 DOI: 10.1086/605476]

31 **Blight K**, Trowbridge R, Rowland R, Gowans E. Detection of hepatitis C virus RNA by in situ hybridization. *Liver* 1992; **12**: 286-289 [PMID: 1333032]

32 **Radkowski M**, Wilkinson J, Nowicki M, Adair D, Vargas H, Ingui C, Rakela J, Laskus T. Search for hepatitis C virus negative-strand RNA sequences and analysis of viral sequences in the central nervous system: evidence of replication. *J Virol* 2002; **76**: 600-608 [PMID: 11752151 DOI: 10.1128/JVI.76.2.600-608.2002]

33 **Forton DM**, Karayiannis P, Mahmud N, Taylor-Robinson SD, Thomas HC. Identification of unique hepatitis C virus quasispecies in the central nervous system and comparative analysis of internal translational efficiency of brain, liver, and serum variants. *J Virol* 2004; **78**: 5170-5183 [PMID: 15113899 DOI: 10.1128/JVI.78.10.5170-5183.2004]

34 **Letendre S**, Paulino AD, Rockenstein E, Adame A, Crews L, Cherner M, Heaton R, Ellis R, Everall IP, Grant I, Masliah E. Pathogenesis of hepatitis C virus coinfection in the brains of patients infected with HIV. *J Infect Dis* 2007; **196**: 361-370 [PMID: 17597450 DOI: 10.1086/519285]

35 **Wilkinson J**, Radkowski M, Eschbacher JM, Laskus T. Activation of brain macrophages/microglia cells in hepatitis C infection. *Gut* 2010; **59**: 1394-1400 [PMID: 20675697 DOI: 10.1136/gut.2009.199356]

36 **Dolganiuc A**, Garcia C, Kodys K, Szabo G. Distinct Toll-like receptor expression in monocytes and T cells in chronic HCV infection. *World J Gastroenterol* 2006; **12**: 1198-1204 [PMID: 16534871]

37 **Sato K**, Ishikawa T, Okumura A, Yamauchi T, Sato S, Ayada M, Matsumoto E, Hotta N, Oohashi T, Fukuzawa Y, Kakumu S. Expression of Toll-like receptors in chronic hepatitis C virus infection. *J Gastroenterol Hepatol* 2007; **22**: 1627-1632 [PMID: 17845690 DOI: 10.1111/j.1440-1746.2006.04783.x]

38 **Peng C**, Liu BS, de Knegt RJ, Janssen HL, Boonstra A. The response to TLR ligation of human CD16+CD14- monocytes is weakly modulated as a consequence of persistent infection with the hepatitis C virus. *Mol Immunol* 2011; **48**: 1505-1511 [PMID: 21531464 DOI: 10.1016/j.molimm.2011.04.008]

39 **Bain C**, Fatmi A, Zoulim F, Zarski JP, Trépo C, Inchauspé G. Impaired allostimulatory function of dendritic cells in chronic hepatitis C infection. *Gastroenterology* 2001; **120**: 512-524 [PMID: 11159892]

40 **Goutagny N**, Fatmi A, De Ledinghen V, Penin F, Couzigou P, Inchauspé G, Bain C. Evidence of viral replication in circulating dendritic cells during hepatitis C virus infection. *J Infect Dis* 2003; **187**: 1951-1958 [PMID: 12792872]

41 **Goutagny N**, Vieux C, Decullier E, Ligeoix B, Epstein A, Trepo C, Couzigou P, Inchauspe G, Bain C. Quantification and functional analysis of plasmacytoid dendritic cells in patients with chronic hepatitis C virus infection. *J Infect Dis* 2004; **189**: 1646-1655 [PMID: 15116301 DOI: 10.1086/383248]

42 **Gelderblom HC**, Nijhuis LE, de Jong EC, te Velde AA, Pajkrt D, Reesink HW, Beld MG, van Deventer SJ, Jansen PL. Monocyte-derived dendritic cells from chronic HCV patients are not infected but show an immature phenotype and aberrant cytokine profile. *Liver Int* 2007; **27**: 944-953 [PMID: 17696933 DOI: 10.1111/j.1478-3231.2007.01507.x]

43 **Ryan EJ**, O'Farrelly C. The affect of chronic hepatitis C infection on dendritic cell function: a summary of the experimental evidence. *J Viral Hepat* 2011; **18**: 601-607 [PMID: 21794024 DOI: 10.1111/j.1365-2893.2011.01453.x]

44 **Palmer BA**, Moreau I, Levis J, Harty C, Crosbie O, Kenny-Walsh E, Fanning LJ. Insertion and recombination events at hypervariable region 1 over 9.6 years of hepatitis C virus chronic infection. *J Gen Virol* 2012; **93**: 2614-2624 [PMID: 22971825 DOI: 10.1099/vir.0.045344-0]

45 **Caussin-Schwemling C**, Schmitt C, Stoll-Keller F. Study of the infection of human blood derived monocyte/macrophages with hepatitis C virus in vitro. *J Med Virol* 2001; **65**: 14-22 [PMID: 11505438]

46 **Royer C**, Steffan AM, Navas MC, Fuchs A, Jaeck D, Stoll-Keller F. A study of susceptibility of primary human Kupffer cells to hepatitis C virus. *J Hepatol* 2003; **38**: 250-256 [PMID: 12586289]

47 **Radkowski M**, Bednarska A, Horban A, Stanczak J, Wilkinson J, Adair DM, Nowicki M, Rakela J, Laskus T. Infection

of primary human macrophages with hepatitis C virus in vitro: induction of tumour necrosis factor-alpha and inter-leukin 8. *J Gen Virol* 2004; **85**: 47-59 [PMID: 14718619]

48   **Laskus T**, Radkowski M, Jablonska J, Kibler K, Wilkinson J, Adair D, Rakela J. Human immunodeficiency virus fa-cilitates infection/replication of hepatitis C virus in native human macrophages. *Blood* 2004; **103**: 3854-3859 [PMID: 14739225]

49   **el-Awady MK**, Tabll AA, Redwan el-RM, Youssef S, Omran MH, Thakeb F, el-Demellawy M. Flow cytometric detection of hepatitis C virus antigens in infected peripheral blood leukocytes: binding and entry. *World J Gastroenterol* 2005; **11**: 5203-5208 [PMID: 16127753]

50   **Ponzetto A**, Gennero L, Cutufia M, Beltramo T, Enrietto M, Pescarmona P, Pugliese A. Effect of HCV infection on THP-1 monocytoid cells. *Cell Biochem Funct* 2005; **23**: 347-352 [PMID: 15515125 DOI: 10.1002/cbf.1158]

51   **Lau DT**, Negash A, Chen J, Crochet N, Sinha M, Zhang Y, Guedj J, Holder S, Saito T, Lemon SM, Luxon BA, Perel-son AS, Gale M. Innate immune tolerance and the role of kupffer cells in differential responses to interferon therapy among patients with HCV genotype 1 infection. *Gastroenterology* 2013; **144**: 402-413.e12 [PMID: 23123437 DOI: 10.1053/j.gastro.2012.10.044]

52   **Negash AA**, Ramos HJ, Crochet N, Lau DT, Doehle B, Papic N, Delker DA, Jo J, Bertoletti A, Hagedorn CH, Gale M. IL-1β production through the NLRP3 inflammasome by hepat-ic macrophages links hepatitis C virus infection with liver inflammation and disease. *PLoS Pathog* 2013; **9**: e1003330 [PMID: 23633957 DOI: 10.1371/journal.ppat.1003330]

53   **Navas MC**, Fuchs A, Schvoerer E, Bohbot A, Aubertin AM, Stoll-Keller F. Dendritic cell susceptibility to hepatitis C vi-rus genotype 1 infection. *J Med Virol* 2002; **67**: 152-161 [PMID: 11992576 DOI: 10.1002/jmv.2204]

54   **Lambotin M**, Baumert TF, Barth H. Distinct intracellular trafficking of hepatitis C virus in myeloid and plasmacytoid dendritic cells. *J Virol* 2010; **84**: 8964-8969 [PMID: 20573834 DOI: 10.1128/JVI.00517-10]

55   **Revie D**, Braich RS, Bayles D, Chelyapov N, Khan R, Geer C, Reisman R, Kelley AS, Prichard JG, Salahuddin SZ. Trans-mission of human hepatitis C virus from patients in second-ary cells for long term culture. *Virol J* 2005; **2**: 37 [PMID: 15840164 DOI: 10.1186/1743-422X-2-37]

56   **Revie D**, Alberti MO, Braich RS, Chelyapov N, Bayles D, Prichard JG, Salahuddin SZ. Analysis of in vitro replicated human hepatitis C virus (HCV) for the determination of genotypes and quasispecies. *Virol J* 2006; **3**: 81 [PMID: 17010197 DOI: 10.1186/1743-422X-3-81]

57   **Revie D**, Alberti MO, Braich RS, Bayles D, Prichard JG, Salahuddin SZ. Discovery of significant variants contain-ing large deletions in the 5'UTR of human hepatitis C virus (HCV). *Virol J* 2006; **3**: 82 [PMID: 17010198 DOI: 10.1186/1743-422X-3-82]

58   **Revie D**, Alberti MO, Prichard JG, Kelley AS, Salahuddin SZ. Analysis of the 5'UTR of HCV genotype 3 grown in vi-tro in human B cells, T cells, and macrophages. *Virol J* 2010; **7**: 155 [PMID: 20626910 DOI: 10.1186/1743-422X-7-155]

59   **Welsch S**, Groot F, Kräusslich HG, Keppler OT, Sattentau QJ. Architecture and regulation of the HIV-1 assembly and holding compartment in macrophages. *J Virol* 2011; **85**: 7922-7927 [PMID: 21613397 DOI: 10.1128/jvi.00834-11]

60   **Farnik H**, Mihm U, Zeuzem S. Optimal therapy in genotype 1 patients. *Liver Int* 2009; **29** Suppl 1: 23-30 [PMID: 19207963 DOI: 10.1111/j.1478-3231.2008.01969.x]

61   NIH Consensus Statement on Management of Hepatitis C: 2002. *NIH Consens State Sci Statements* 2002; **19**: 1-46 [PMID: 14768714]

62   **Di Liberto G**, Roque-Afonso AM, Kara R, Ducoulombier D, Fallot G, Samuel D, Feray C. Clinical and therapeutic impli-cations of hepatitis C virus compartmentalization. *Gastroenterology* 2006; **131**: 76-84 [PMID: 16831592]

63   **Sansonno D**, Tucci FA, Lauletta G, De Re V, Montrone M, Troiani L, Sansonno L, Dammacco F. Hepatitis C virus pro-ductive infection in mononuclear cells from patients with cryoglobulinaemia. *Clin Exp Immunol* 2007; **147**: 241-248 [PMID: 17223964]

64   **Boddi M**, Abbate R, Chellini B, Giusti B, Giannini C, Pra-tesi G, Rossi L, Pratesi C, Gensini GF, Paperetti L, Zignego AL. Hepatitis C virus RNA localization in human carotid plaques. *J Clin Virol* 2010; **47**: 72-75 [PMID: 19896417]

65   **Kurokawa M**, Hidaka T, Sasaki H, Nishikata I, Morishita K, Setoyama M. Analysis of hepatitis C virus (HCV) RNA in the lesions of lichen planus in patients with chronic hepati-tis C: detection of anti-genomic- as well as genomic-strand HCV RNAs in lichen planus lesions. *J Dermatol Sci* 2003; **32**: 65-70 [PMID: 12788531]

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