

Review article

Virus persistence in hepatitis C: lifelong infection despite therapy?

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RESUMEN

La infección crónica por virus de la hepatitis C (VHC) es un problema creciente a nivel mundial y constituye actualmente la causa más frecuente de trasplante hepático en Estados Unidos y Europa. El tratamiento actual consiste en la combinación de interferón pegilado y ribavirina, con respuesta viral sostenida en más del 50% de los pacientes. En la actualidad existen pruebas que sustentan el concepto de infección oculta o persistente por VHC en el hígado y las células mononucleares de sangre periférica en sujetos con resolución espontánea o farmacológica, definida convencionalmente como ausencia de viremia al menos seis meses después del tratamiento. Este fenómeno podría tener relevancia clínica, pues los sujetos con infección persistente pudieran estar en riesgo de recurrencia de la infección o de aparición de manifestaciones extrahepáticas que resultarían en un pronóstico desfavorable. Este artículo tiene como objetivo revisar la bibliografía actual al respecto de la persistencia viral y la infección oculta y sus potenciales implicaciones clínicas.

Palabras clave: virus hepatitis C, enfermedad hepática, células mononucleares sanguíneas periféricas, infección oculta, hepatitis viral, viral persistente.

ABSTRACT

Chronic hepatitis C virus (HCV) infection is a common and growing problem in the world and is currently the most common reason for liver transplantation in the United States and Europe. Current therapy includes a combination of pegylated interferon and ribavirin, which has been shown to produce a sustained viral response in greater than 50% of patients. There is increasing evidence that supports the concept of occult or persistent HCV infection within hepatocytes and peripheral blood mononuclear cells (PBMCs) after spontaneous or therapy-induced sustained viral response defined as absence of detectable viremia at least 6 months after end of therapy. This may have some clinical importance as patients with persistent HCV may have increased risk of recurrence or extrahepatic manifestations of the infection that will alter their ultimate prognosis. Therefore, this article serves as a review of the current literature in HCV persistence in both hepatic and extrahepatic sites and their clinical implications.

Key words: hepatitis C virus, liver disease, peripheral blood mononuclear cells, occult infection, viral hepatitis, viral persistence.

Hepatitis C virus (HCV) is a commonly encountered pathogen in medical practice. It is estimated that 2-3% of the world is affected by HCV, with a prevalence of 170 million people (3% of the world's population) and incidence of 3-4 million per year.¹ In the general United States population, its prevalence is 1.8%, affecting approximately 2.7 million people.² Transmission of the virus occurs by injection drug use, blood transfusion prior to 1992 when blood was not

screened for the virus, and rarely by sexual transmission.³ Most (50-75%) of those affected with the virus develop chronic hepatitis, defined as detection of HCV RNA for greater than 6 months. Approximately 20% of those with chronic hepatitis further develop cirrhosis and 1-4% of cirrhotic patients will go on to develop hepatocellular carcinoma.^{3,4} Importantly, HCV is the most common etiologic factor for liver transplantation in the United States and Europe, where up to 35% of liver transplant candidates are infected with HCV.^{5,6}

HCV is a RNA virus of the *Flaviviridae* family and *Hepacivirus* genus. It is a linear, single-strand, positive-sense, RNA genome of approximately 9600 nucleotide bases.^{4,7} The presence of negative-strand HCV RNA is generally believed to be indicative of active viral replication, as single-stranded RNA viruses replicate by synthesizing a negative-strand RNA molecule from the positive strand, which then acts as a template for production of the genomic, positive strand RNA virus. There are

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6 genotypes of HCV, with the most common genotypes in the United States being genotype 1 (75%), genotype 2 (15%), and genotype 3 (7%). Response rates to treatment are lowest in genotype 1.³ Diagnosis of HCV is based on serologic detection of antibodies to the virus, confirmed by detection of the RNA in the serum by diverse molecular techniques.

Current therapy consists of the combination of interferon alpha (pegylated interferon) and ribavirin. Interferon alpha is a cytokine that has innate antiviral immune responses by a proposed pathway of inducing the expression of genes that interfere with replication of HCV (double-stranded RNases, inhibitors of viral protein translation, and proteins that destabilize the viral messenger RNA) and those that activate the immune response. Pegylated interferon (peginterferon) is a long-acting form of interferon which can be administered weekly. Ribavirin is a nucleoside analogue whose pathophysiology against HCV is not entirely clear, but believed to be secondary to immune modulation, decreasing intracellular guanosine triphosphate needed for viral RNA synthesis, and/or mutation of virions. The combination of the two drugs produces a sustained viral response (defined as the absence of serum HCV RNA for more than 6 months after therapy, when tested with highly sensitive assays) in 54-56% of patients, compared to less than 20% with interferon alpha alone, 29% with peginterferon alone, and 44-47% with standard interferon and ribavirin combined.³ In end-stage HCV-related liver disease, liver transplantation is the only treatment that can improve patients' prognosis.⁵

Recent studies demonstrate that HCV may persist in some body compartments despite spontaneous clearance or sustained viral response after antiviral therapy, and result in occult infections. There has been evidence to suggest that viral RNA persists in the serum, liver and peripheral blood mononuclear cells (PBMCs) when sensitive assays are used in patients that fit the conventional criteria for sustained viral response.⁸ Therefore, this article serves as a review of the evidence for and against occult and persistent HCV infections and the potential clinical implications that this phenomenon may have.

EVIDENCE FOR OCCULT INFECTION IN THE LIVER

Occult HCV infection is defined as the presence of HCV RNA in the liver, but absence of antibodies to HCV and

HCV RNA in the serum.⁹ McHutchison et al¹⁰ studied whether viral persistence was found in the liver after treatment-induced sustained viral response. This was a large trial that examined 2,089 patients who had chronic hepatitis C that was treated with interferon alpha-2b with or without ribavirin for 24 to 48 weeks. The subjects had a liver biopsy performed (951 patients) before and 24 weeks after receiving the treatment (1,316 patients). The majority (903 of 951, or 95%) of the pretreatment liver biopsies contained detectable HCV RNA by a method with high sensitivity (1 viral copy per mcg of total cellular RNA). The pretreatment hepatic HCV RNA levels reflected serum HCV RNA levels and were inversely proportional to the degree of liver parenchymal injury and fibrosis. Nine hundred sixteen of the 1,316 biopsies taken after treatment were from nonresponders, and 850 (93%) had detectable and 66 (7%) had undetectable hepatic HCV RNA. The remaining 400 liver biopsies performed after therapy were from patients with sustained viral response, and of these patients 393 (98%) had undetectable and 7 (2%) had detectable hepatic HCV RNA. Of the 7 with detectable HCV RNA, 2 relapsed with positive serum HCV RNA within 12 months after treatment was completed, while the others had sustained viral response either at 3.5 years, 12 months, and 24 weeks. Previous smaller studies such as Shindo et al¹¹ provided evidence that hepatic viral clearance correlated with sustained viral response, and this study found that persistent HCV RNA in the liver was rare but was associated with relapse.

In a later investigation performed by Radkowski et al¹² 17 randomly chosen patients with sustained viral response after interferon alpha-2b with (12) or without (5) ribavirin therapy were studied for viral persistence in the liver and PBMCs. The controls were 15 healthy subjects who did not have antibodies to HCV throughout the study period. These patients were followed for 4-9 years. Eleven of the 17 patients had follow-up liver biopsies all of which had improvement in necroinflammatory changes and the majority (9 of 11) had improvement in liver fibrosis scores. There were 3 patients with detectable HCV RNA, and interestingly the 2 without improvement in liver fibrosis were part of this group. None of the 3 subjects had detectable negative-strand HCV RNA present in the liver. Their conclusion was that there is viral persistence, but not necessarily replication, in the liver of those patients with sustained viral response to treatment.

Castillo et al¹³ took a different approach to determine the presence of occult HCV infection. They examined 100 patients with persistently elevated liver-function tests of unknown etiology for the presence of HCV RNA in liver biopsies. Based on epidemiological and clinical data, the patients were excluded if they had hepatitis B infection, serological evidence of HCV infection, autoimmunity, alcohol intake, metabolic and genetic disorders, HIV, and drug toxicity. The presence of HCV RNA in the liver biopsies was tested by RT-PCR (reverse transcription-polymerase chain reaction) and *in situ* hybridization and was positive in 57 of the 100 patients (57%). Of these individuals, 48 (84.2%) had detectable negative strand HCV RNA, suggesting ongoing viral replication. Interestingly, the study also looked at HCV RNA in PBMCs, and 40 of the 57 (70%) were positive by both RT-PCR and *in situ* hybridization. Interestingly, all of the patients with the detectable HCV RNA had genotype 1b. In relation to the pathological findings in these subjects, they had significantly higher necroinflammatory and fibrotic changes and lower steatosis changes compared to those patients in whom HCV RNA was not detected in the liver biopsies. Therefore this group of investigators provided evidence that patients with elevated liver-function tests of unknown etiology may have occult HCV infection.

An important distinction to make is between occult infection and chronic infection with HCV. Pardo et al¹⁴ compared clinical and pathological characteristics of 68 patients with occult HCV infection and 69 patients with untreated chronic HCV. The defining difference between these patients was that those with chronic infection had positive serological tests for HCV antibodies and HCV RNA. The groups were matched for age, gender, body mass index, and estimated duration of abnormal liver-function tests. For clinical laboratory parameters, alanine aminotransferase, gammaglobulin, alpha-fetoprotein, and iron levels were significantly higher in patients with chronic HCV infection, while triglycerides and cholesterol levels were significantly higher in patients with occult HCV infection. No significant difference was found for aspartate aminotransferase, gamma-glutamyl transpeptidase, and ferritin. Regarding histological findings in the liver biopsy, those with chronic infection were found to have significantly higher rates of necroinflammatory activity and fibrosis than those with occult infection. There was no significant difference between the rates of steatosis in the

2 groups. Genotype 1b was found in all the liver biopsies taken of those with occult infection, while only in 52 of the 65 with chronic HCV infection that had a liver biopsy (80%). As expected, the percentage of HCV-infected hepatocytes was significantly higher in those with chronic infection, which could account for the greater liver damage in this group. All of this provides evidence that occult HCV infection is a milder disease, which may be explained by the difference in cellular immune responses between the 2 groups described in an earlier study by Quiroga et al.¹⁵ This study compared 50 patients with occult HCV infection, 141 with chronic HCV, and 21 with cryptogenic liver disease (HCV RNA undetected in both serum and liver). Those with occult infection had more frequent HCV-specific CD4⁺ and CD8⁺ T cell responses in the peripheral blood, with the magnitude of responses correlating with the extent of liver infection. In addition, the HCV-specific T cells in those with occult infection proliferated more readily in response to HCV NS3 and NS4 envelope proteins. With these findings, it was concluded that those with occult infection have better immunologic responses to the virus, possibly accounting for the milder disease seen.

EVIDENCE FOR EXTRAHEPATIC PERSISTENCE AND REPLICATION

Peripheral blood mononuclear cells (PBMCs)

PBMCs are the most studied extrahepatic site for HCV infection. In the early 1990s, the first studies to find evidence of replication of HCV in PBMCs were published, although they were performed on patients with chronic HCV infection, rather than patients with sustained viral response. Within the PBMCs subgroup, T-cell and B-cell lymphocytes and monocytes/macrophages have been shown to harbor HCV infection.¹²

Evidence for persistent HCV infection in patients with sustained viral response, either by treatment or spontaneously, was first reported by Phan et al in 2004.¹⁶ Sixteen randomly selected patients with consistently normal aminotransferases and negative serum HCV RNA were studied, 5 with spontaneous resolution and 11 with treatment-induced resolution (10 with standard interferon and ribavirin combination therapy and 1 with standard interferon alone). A highly sensitive method was used to detect the HCV genome in the serum and PBMCs for up to 60 months after resolution. Although the patients

were repeatedly negative for HCV RNA when tested using the standard clinical laboratory RNA isolation and RT-PCR amplification, the investigators found that 15 of the 16 patients (4 of the 5 with spontaneous and all 11 of those with treatment-induced resolution) had persistent presence of the genome when using a highly sensitive RT-PCR-NAH (reverse transcription-PCR-nucleic acid hybridization) assay (sensitivity <10 viral genome equivalents/ml, which is at least 10-fold more sensitive than the standard current laboratory tests). Mitogen stimulation of PBMCs with IL-2 (cytokine essential for T-cell growth) and phytohemagglutinin (PHA; potent nonspecific inducer of T-cell proliferation) enhances the replication of the residing HCV and therefore thought to allow easier discovery. With mitogen-stimulated PBMCs, 13 of the 16 patients (81%) had measurable positive-strand HCV RNA, while in non-stimulated PBMCs, only 3 of 9 (33%) were reactive. Interestingly, all of the patients with sustained viral response had HCV RNA in either the sera and/or PBMCs. Furthermore, negative-strand RNA was detected in the majority (75%) of mitogen-stimulated PBMCs. Therefore, by using a very sensitive assay, it was found that HCV genomes persist for up to 60 months in the serum and circulating PBMCs in patients who were originally thought to have sustained viral response, either spontaneously or with treatment.

In a similar investigation described above, Radkowski et al¹² looked at the persistence of HCV after sustained viral response with treatment. Overall, 15 of the 17 patients (88%) had evidence of HCV RNA either in the liver, serum, or PBMCs. A total of 9 of the 17 patients (53%) were HCV RNA positive in unfractionated PBMCs after 2-3 collections. After mitogen-stimulation of the PBMCs by PHA, 14 of the 17 (82%) were positive for HCV RNA after 2-3 collections over time, providing evidence that mitogen stimulation increases the replication of HCV RNA allowing for better detection. Altogether, there were 29 PBMC samples collected that were HCV RNA positive, and of these 6 (21%) had detectable negative-strand HCV RNA. This study enhanced the findings that occult infections of the serum or PBMCs occur in patients with sustained viral response, and the detection of HCV RNA may be aided with repeated testing.

Since both of these studies used patients treated with standard interferon therapy with or without ribavirin rather than the currently accepted combination treatment with

peginterferon and ribavirin, Gallegos-Orozco et al⁸ studied whether the proven more effective form of therapy had an effect on the persistence of HCV RNA in PBMCs. Twenty-five patients with sustained viral response to combination therapy with peginterferon alfa-2a and ribavirin were investigated. The duration of treatment varied according to the specific genotype infecting the patient (48 weeks for genotype 1 versus 24 weeks for genotype 2 and 3). A highly sensitive RT-PCR was used (sensitivity <10 copies/ml) on serum and mitogen-stimulated PBMCs. Persistent HCV infection, with HCV RNA detected in PBMC cultures, was present in 5 of the 25 patients (20%), 3 patients with genotype 1 and 2 patients with genotype 2. This percentage is significantly lower than that described in patients with sustained viral response to standard interferon alone or in combination with ribavirin, as well as in subjects with spontaneous viral clearance, which may be attributed to the fact that the patients in this study were treated with the most current regimen and had shorter follow-up after the end of treatment.

Although the above studies provided evidence for the presence of HCV RNA in PBMCs, they did not focus on the replicative properties of the virus. Castillo et al⁹ studied 18 patients with occult HCV infection to determine the ability of HCV to replicate within PBMCs. All 18 patients had HCV RNA positive-strand detected in PBMCs, while 11 of the 18 (61%) had HCV RNA negative-strand detected by specific RT-PCR and confirmed by fluorescence *in situ* hybridization (FISH) analysis. This percentage of replicative activity in patients with occult infection is similar to that reported in PBMCs of patients with chronic hepatitis. The percentage of replicative HCV in PBMCs in this study is slightly lower than that in the Phan et al. study above, but both reports demonstrate that the majority of people with persistent HCV infection have replicative ability in PBMCs. However, there was no correlation between the presence of HCV replication in hepatocytes and PBMCs. The ability to detect negative-strand RNA may be affected by the fact that there usually is less amounts of negative-strand present as a whole, therefore making the sensitivities of the detection techniques lower than that for positive-strands. In one study, the ratio of positive-to-negative-strand ranged from 2.1 to 11.3 (mean 6.6) in PBMC samples, which is lower than the ratio reported for the liver but similar to HCV-infected macrophage cell cultures.¹²

The above evidence for PBMC persistence and replication of HCV has been challenged by other investigators. In 1997 Laskus et al¹⁷ described 27 patients with chronic hepatitis C, none of whom received any antiviral therapy prior to the study. They used *Tth*-based (a thermostable enzyme thought to decrease the rate of mispriming; strand specific) or MMLV-based (Moloney murine leukemia virus; standard, not strand specific) RT-PCR to detect HCV-RNA, both positive- and negative-strands, in PBMCs and serum. In addition, assays for 2 sites, the 5' untranslated (5'UTR) and NS5 (non-structural protein 5) coding regions, were tested. Using the MMLV assay on PBMC samples, 17 of the 5'UTR and 12 of the NS5 region returned with positive-strand HCV RNA. In addition, all 12 patients who had detectable positive-strand HCV RNA in the PBMCs with primers specific for the NS5 region, also had the same region positive in the serum when the amplified sequences were analyzed by single strand conformation polymorphism (SSCP). PBMCs and serum from all 27 patients were negative for the negative strand RNA when using the *Tth*-based assay. Therefore, this study suggested that there was no extrahepatic replication occurring. Furthermore, because the same HCV RNA sequences were found in both PBMCs and serum of the 12 patients that had detectable positive-strand RNA in the PBMCs, it was thought that the presence of the HCV RNA in PBMCs was either a contamination by the circulating virus or passive viral adsorption rather than actual extrahepatic replication. It should be noted that this study was performed on patients that did not have occult infection, but instead had chronic HCV infection in which treatment was not undertaken prior to the study. Also, even though the assays used were highly sensitive and specific, they did not utilize mitogen-stimulated PBMCs, a technique that was later found to enhance the detection of HCV RNA in PBMCs.¹⁶

A recent study by Bernardin et al¹⁸ provided more evidence against HCV persistence and replication in PBMCs. The subjects in this study were identified from lists of voluntary blood donors who were found to be HCV-seropositive. Initially, the plasma viremia status of the subjects was determined using a highly sensitive transcription-mediated amplification (TMA) assay, 67 patients were aviremic and 58 were viremic on their initial blood donation. At an average of 2.5 years later, the patients' viremic status was retested and 60 of the initial 67 aviremic

and 9 of the initial 58 viremic patients did not have detectable serum HCV RNA; furthermore, all of these patients also were negative for HCV RNA in PBMCs via the highly sensitive CA-TMA and RT-nPCR assays. A majority of those with viremia (43 of 56, 77%), whether treated or not, had HCV RNA detected in their PBMCs, especially if the viral load was high with persistent positive TMA assay. Their conclusions were that PBMCs were most likely not a long-lived reservoir for HCV, but instead reflected the plasma HCV content. Several factors may have played a role in these observations, including the fact that the PBMCs were not mitogen-stimulated therefore could have false negative results and their definition of viremia was more stringent using the TMA assay with a 95% detection limit of 30 RNA copies/ml with duplicate testing. These findings were similar to an earlier study that concluded that there was no evidence for persistent HCV infection in PBMCs if viremia was cleared in the 9 of 30 subjects with HCV and HIV co-infection.¹⁹ Furthermore, in 3 patients that experienced spontaneous or treatment-induced sustained viral response during that study, the HCV RNA in the PBMCs also became undetectable. Therefore, these 2 studies question whether PBMC persistence of HCV is of clinical significance.

Central nervous system (CNS)

Several studies have also looked at whether the CNS is a site of extrahepatic replication of HCV. Radkowski et al²⁰ looked for negative-strand HCV RNA in 6 patients with known active HCV infection, 5 with cirrhosis and 3 with HIV co-infection, at autopsy. Of these patients, the cause of death was sepsis (1), liver failure (2), drug overdose (2), and acute pancreatitis (1). Brain tissue (subcortical white matter and cerebral cortex from frontal region, nucleus lentiformis, cerebellum, and medulla oblongata) was analyzed in all 6 patients and mediastinal lymph node tissue in 4. *Tth*-based RT-PCR was used for the detection of the negative-strand HCV RNA. In 3 patients, only 1 with HIV co-infection, negative-strand HCV RNA was detected in the medulla oblongata, cerebellum, or subcortical white matter. In the patient with positive cerebellar HCV RNA, the mediastinal lymph nodes were also positive for negative-strand HCV. No patient had negative-strand RNA present in the serum. Negative-strand RNA titers in the brain tissue were 1 log lower than positive-strand titers, similar to that found in the liver. In 2 of the 3 patients

with negative-strand RNA in the brain tissue, the viral sequences were different from those derived from the serum by SSCP assay, while the patient with both brain and lymph node negative-strand RNA had identical sequences in the two tissues. Genotypes also differed between the serum and CNS tissues of those 2 patients with differing sequences, as both had genotype 1b in the serum and either genotype 3a or 1a in the CNS, suggesting either a coinfection or superinfection with different strains. To further determine whether PBMCs were a significant source of the negative-strand RNA, mRNA phenotyping by RT-PCR of CD2 (T cells), CD19 (B cells), and CD14 (monocyte/macrophage) were performed on the brain tissue of the 3 patients that had detectable negative-strand HCV RNA. Expression of CD14 was present in all 3 samples, CD19 in none, and CD2 had a low titer in 1. Therefore, with the presence of negative-strand RNA in the brain with differing viral sequences, the authors inferred that HCV replicates in the CNS, most likely through the trafficking of infected cells of the monocyte/macrophage lineage. Because of the similar viral sequence found in the CNS and lymphatic system in 1 patient, there was further evidence of the lymphoid origin. However, it must be taken into consideration that this study was performed on patients that had active rather than occult or persistent infection.

The above study had evidence of persistence and replication within the brain matter in patients with active chronic HCV infection with no evidence of CNS involvement, however to take it a step further, Vargas et al²¹ studied patients with known psychiatric symptoms. To determine the possibility of whether the CNS is a source of extrahepatic replication in patients that had recurrence of HCV after liver transplantation and severe depression, they looked at 2 cases, 1 that died from multiorgan failure and the other from *S. aureus* septicemia. Brain tissue samples at autopsy were collected from the subcortical white matter and cerebral cortex of the frontal region and brainstem from both patients, and cerebellum from 1 patient. Both patients had positive HCV RNA in the serum and all brain tissues sampled using RT-PCR. In addition, using *Tth*-based RT-PCR assay, negative-strand RNA was detected in the subcortical white matter of 1 patient and the cerebral cortex of the other. Using SSCP assay to determine the viral sequences, it was shown that the positive-strand RNA was similar to the serum viral sequences, while the negative-strand RNA was different. This discrepancy was

explained by the fact that the positive-strand RNA most likely represented a serum-derived contamination, while the negative-strand RNA represented indigenous replicating virus within the brain matter. These results raise the question of the potential role for viral replication in the CNS of patients with chronic hepatitis C and the psychiatric symptoms they commonly present with and that tend to improve after successful antiviral therapy.

While the above studies looked at the brain tissue itself, Bagaglio et al²² investigated the cerebrospinal fluid (CSF) of 21 patients with HIV and HCV co-infection. They specifically looked at HIV patients as they more commonly have HCV RNA in PBMCs and mixed infections with different genotypes, likely related to their immunocompromised state. Five of the 21 (24%) had detectable HCV RNA in the CSF, independent of a statistically significant association with plasma HCV and HIV viral loads. The 5 patients did have higher HIV RNA levels in the CSF, suggesting that HIV entry into the CSF may allow for facilitated transmission of HCV. This is supported by the fact that 3 of these patients also had high levels of cells and protein in the CSF, indicating that the blood-brain barrier was disturbed, allowing easier neuroinvasion. Furthermore, by viral sequencing, it was found that 2 different genotypes were present in the CSF and plasma/PBMCs (genotype 1b in CSF versus genotype 3a in plasma/PBMC in 2 patients). This raises the question of coinfection or superinfection with different strains of HCV, and independent replication in different body compartments.

CLINICAL IMPLICATIONS

Source of recurrence in immunodeficient states

A case study by Lee et al²³ brought up the possibility that persistent HCV RNA in the serum or PBMCs may be a source of recurrent HCV infection in patients with immunocompromised or immunosuppressed states. The patient was a white female who developed HCV after receiving contaminated intravenous gamma globulin (IVIG) for IgG immunodeficiency. She was treated with interferon alpha-2b for approximately 4.5 months, stopped treatment when her HCV RNA was undetectable, immediately relapsed, and then spontaneously reached a sustained viral response. For 9 years, the patient did not have any detectable HCV RNA in the serum, even while receiving routine IVIG treatment. Only when the patient

was given methylprednisolone with IVIG infusions and had several courses of prednisone for asthmatic episodes, did she develop a recurrent hepatitis and was found to have HCV RNA in the serum. The virus spontaneously cleared within 2 months of discontinuation of the corticosteroids. Testing from the frozen serum of her previous episodes confirmed that the recurrent infection was of the same genotype 1 virus. This study suggests that occult infection may be the source of HCV reactivation during immunocompromised states.

Recurrence of HCV infection after liver transplantation

Liver transplantation with subsequent immunosuppressive treatment can possibly be of concern since the case study above showed that HCV may recur in patients who are immunocompromised. The natural history of HCV after liver transplantation has been studied and there is evidence that the reinfection rate after transplantation is almost 100% [6]. Laskus et al⁷ studied whether recurrence of HCV infection after liver transplantation was affected by the presence of HCV RNA in PBMCs or serum. They studied 6 patients who had orthotopic liver transplantation for end-stage HCV-related cirrhosis that received a graft from a HCV-negative donor, determined by the absence of anti-HCV in the serum and HCV RNA by RT-PCR in the liver. All 6 of these patients had reinfection by HCV. It was found that in 2 of the 6 patients, the source could not be determined since the PBMC and serum-derived viral sequences were the same. In 3 of the remaining 4 patients, the viral sequence of the post-transplant serum matched that of the pretransplant serum sequences, rather than the PBMC sequences. This suggested that the liver graft was reinfected by the virions left in circulation after transplant rather than an extrahepatic source.

Relationship to hepatocellular carcinoma

Since hepatocellular carcinoma (HCC) is the most feared consequence of chronic hepatitis C infection, it is important to determine the relationship between occult HCV infection and HCC. A case report by Esaki et al²⁴ described a patient with HCC that had negative serum anti-HCV antibody and HCV RNA, but positive liver tissue HCV RNA. Although the negative serological studies may be attributed to false negatives, the patient had repeat anti-HCV antibody and HCV-RNA testing in

the serum 3 months later, which were once again negative, decreasing the chance of an error in testing. This patient was diagnosed with liver dysfunction 10 years earlier, but according to the authors was not further worked up, therefore the natural history of the disease is unknown in this patient. However, this is the first report of occult hepatitis C associated with HCC.

In a retrospective study by Comar et al²⁵ also provided evidence that occult HCV infection can be present in people with HCC. They took 8 patients with HCC and performed liver biopsies to look for the presence of HCV, hepatitis B virus (HBV), and transfusion transmitted virus (TTV) in comparison with serology markers. None of the patients had been treated with antiviral medications prior to the biopsies. The authors used *in situ* PCR to enhance the sensitivity of detecting the viruses in the liver. Focusing on only the HCV results, there were 5 patients with detectable HCV RNA in the liver biopsies, but only 3 of these patients had the presence of HCV antibodies and RNA in the serum. Therefore, the other 2 patients had evidence of occult infection with HCV in liver biopsies of HCC.

Hematologic diseases associated with HCV

Hepatitis C has been associated with extrahepatic manifestations, including skin, renal, hematologic, and rheumatologic systems. Mixed cryoglobulinemia, Hodgkin's and non-Hodgkin's lymphoma (NHL) are the blood disorders found to be associated with HCV. On a study using the Department of Veterans Affairs database of patients with HCV infection hospitalized between 1992-1999, the association between HCV and these disorders were undertaken. Cryoglobulinemia was found to be 11 times more frequent in patients with HCV than controls. There was no significant difference between the two groups in regard to NHL, however, it did become significant when adjusted for age.²⁶ Although these results are in patients with chronic hepatitis C rather than occult infection, it may be that patients with occult HCV may be predisposed to these conditions.

Neurologic diseases associated with HCV

In addition to the extrahepatic systems involved in HCV infection mentioned above, there has also been evidence to suggest that the neurologic/psychiatric systems are also affected. Patients with chronic hepatitis C score worse on health-related quality of life indices than matched controls,

and improvement occurs with successful antiviral therapy.²⁷ Chronic HCV is also associated with chronic fatigue syndrome, impaired memory, and psychiatric symptoms. To study this concept, Forton et al²⁸ randomly selected 30 patients with mild chronic hepatitis C infection. Controls were patients that were either healthy or had chronic hepatitis B infection. All subjects underwent a cerebral proton magnetic-resonance spectroscopy (MRS), and those with HCV had elevations of choline/creatinine ratios in the basal ganglia and white matter, independent of hepatic encephalopathy symptoms or history of IV drug abuse. This is similar to what was found in patients with HIV infection. Therefore, this study supports the concept of brain involvement of HCV and its potential contribution to the psychiatric and neurologic symptoms that are frequently associated with chronic hepatitis C.

AREAS OF UNCERTAINTY

Although there have been many advances in the detection of HCV infection, both in hepatic and extrahepatic sites, there still remains controversy over the clinical significance of these findings and whether they actually represent virus persistence and replication. It is not known whether the detection of HCV RNA in extrahepatic sites directly correlates with persistence and infectivity, rather than random findings with no clinical significance. Most of the clinical implications are implied rather than actually proven and investigators have focused on chronic hepatitis rather than occult infection.

As expected, the methods used to determine HCV persistence have varied between studies, especially since there are different techniques to detect the presence of HCV RNA. Technically the different assays used for detection should not be the source of variability, as the majority of these assays are both highly sensitive and specific. Some studies have found that mitogen-stimulation of PBMCs help with detection of HCV RNA. Therefore, those studies that have used mitogen-stimulation have typically found HCV RNA in PBMCs, while those that did not, both detected and did not detect the presence of HCV RNA in PBMCs. Hopefully, with the evidence that mitogen-stimulation may increase the reliability of the assay to detect HCV RNA, more studies will be conducted with mitogen-stimulation to lessen this area of inconsistency. Another area where the methods differ between studies is

the use of varying treatment methods. Since most studies have been performed prior to the current recommendations of peginterferon with ribavirin for treatment of HCV, they may have had more difficulty attaining sustained viral response with the older therapies. If additional investigations are undertaken with the current recommendations, there may be increased chances of similar results.

Another area of uncertainty is the role that HCV genotype plays in viral persistence in extrahepatic sites. One would assume that genotype 1b would have the highest association with extrahepatic replication, but to date this has not been studied. In addition, in the studies of occult infection reported above, it was found that those with occult infection all had genotype 1b. This would suggest that this genotype is associated with occult infection, but more cases of occult HCV infection will have to be genotyped before this is proven.

CONCLUSION

Hepatitis C is an important pathogen in medical practice, especially since the natural history of the majority of infected patients includes chronic hepatitis and possibly cirrhosis and hepatocellular carcinoma. Current treatment includes a combination of pegylated interferon and ribavirin. However, there is increasing evidence to suggest that even with treatment, the virus may persist in both hepatocytes and extrahepatic sites, including PBMCs and the central nervous system, in the absence of serologic evidence of HCV. Furthermore, investigators have the ability to detect negative-strand HCV RNA, which has been used as a marker for active viral replication. Negative-strand RNA has been found in PBMCs and hepatocytes in patients with negative serological markers, but has not been studied in the central nervous system. Although this topic remains controversial, the development of more sensitive assays and methods (mitogen-stimulated PBMCs) for the detection of the presence of HCV RNA and antibodies will allow for decreased variation between methods used by investigators and possibly a final answer in the future.

The clinical significance of viral persistence and occult infection has also been studied, but not to the extent to find a causal relationship between persistence and the development of HCC, hematologic, or neurologic disorders. Further studies are needed to determine the infectivity and significance of occult infection, especially whether it can

cause recurrence of HCV and lead to diseases associated with chronic infection. Since HCV is the most common etiologic factor related to liver transplantation, it has been suggested that HCV recurrence can be related to persistence of infection in extrahepatic sites, but this will also need to be further studied. Therefore, there are many areas that need further investigation to determine the clinical relevance of HCV occult or persistent infections.

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