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Is HCV Infection a Neurologic Disorder?

See "Hepatitis C virus infects the endothelial cells of the blood-brain barrier," by Fletcher NF, Wilson GK, Murray J, et al, on page 634.

Hepatitis C is known to induce chronic hepatitis, cirrhosis, and hepatocellular carcinoma. The absence of symptoms is common even when the disease has reached the stage of cirrhosis or hepatocellular carcinoma. For this reason, infection with hepatitis C virus (HCV) is frequently called a "silent killer." Fatigue is the most frequent complaint in infected subjects. HCV infection has also been associated with cognitive dysfunction and depression, which are not correlated with the severity of liver disease and cannot be explained by hepatic encephalopathy or drug abuse.

Numerous extrahepatic disorders have been attributed to or are more frequent during HCV infection. The most common mechanism underlying these disorders is autoimmunity. Thyroiditis, arthropathies, lymphocytic sialadenitis with or without sicca syndrome, and diabetes have long been known to be more frequent with HCV.[1,2] The second most frequent disorder associated with HCV infection is type II or III cryoglobulinemia. Cryoglobulins contain immune complexes made of HCV virions and anti-HCV antibodies and are highly prevalent in infected subjects. This can lead to symptomatic vasculitis with purpura, arthralgias, and asthenia, as well as peripheral nervous system and kidney involvement. Cryoglobulins are considered to be the result of B-cell proliferation owing to chronic antigenic stimulation, and B-cell lymphomas have been reported to be slightly more common in this population.[3] A direct role of HCV is suggested by the fact that antiviral therapies have an antitumoral effect.[4] The third group of extrahepatic symptoms associated with HCV infection could be a direct consequence of HCV infection of components of the central nervous system. Indeed, HCV RNA has been detected at autopsy in brain tissues.[5–8] Recently, an elegant study using microdissection in brain tissues from HCV-positive patients obtained at autopsy combined with measurement of cytokine mRNA in cells that were positive for the HCV nonstructural 3 protein suggested that brain macrophages/microglia cells were activated in HCV-infected patients.

The purpose of the paper by Fletcher et al,[9] published in this issue of GASTROENTEROLOGY, was to explore the original hypothesis that HCV could infect and alter the function of blood–brain barrier (BBB) endothelial cells. The authors first demonstrated that all of the known viral receptor molecules (CD81, claudin-1, occludin, LDLR, and scavenger receptor-B1) are expressed at the surface of BBB endothelial cells. It is important to note that scavenger receptor-BI expression was restricted to the microvascular endothelium, whereas other receptors were expressed by astrocytes. In a second step, the authors convincingly showed that HCV replicates in 2 distinct cell lines derived from these BBB endothelial cells. Hepatitis C is known to infect nonhepatocyte cells. There are numerous reports in the literature describing the presence of HCV RNA in immune cells[10] as well as in the brain.[11] In immune cells (mainly B-cells)[12] and in the central nervous system,[13,14] the detection of viral sequences different from those found in the blood or the liver illustrates the compartmentalization of viral quasispecies and supports the idea that replication

takes place in these extrahepatic sites. Indeed, in the absence of local replication, the presence of HCV RNA would be because of the adsorption or internalization of circulating viral particles produced in the liver; thus, no phylogenetic differences would exist with majority variants present in the serum (unless selection of liver-generated viral variants takes place at the cell receptor level). In almost all studies, the level of extrahepatic replication of HCV has been reported to be low, so that the contribution of these sites to circulating virions is limited. The HCV genome is positively stranded, meaning that it is directly translated into the viral polyprotein in the cell cytoplasm. The detection of negatively stranded HCV RNA in tissues or cells demonstrates that viral proteins have been translated and the HCV replication complex is functional, at least for the synthesis of negatively stranded HCV RNA. This intermediate of replication has frequently been detected in extrahepatic sites. There are, however, numerous technical issues that challenge the specificity of such detection. The detection of viral proteins in tissues or cells is also difficult because of their low level of expression. However, their presence has been convincingly reported recently in lymph nodes from the liver pedicle[15] and in the brain.[16]

The most important information in the article by Fletcher et al[9] is that human cell lines derived from BBB endothelial cells can be infected by HCV. The authors first tested HCV binding using HCV pseudoparticles (HCVpp) formed by the incorporation of the envelope glycoproteins E1 and E2 into lentiviral core particles. HCVpp closely mimic the functionality of wild-type viruses during the early steps of the viral lifecycle. HCVpp binding to endothelial cells was quantitatively similar to that observed with hepatocyte cell lines. The authors then used the JFH1 strain, the only HCV strain that effectively infects and replicates in primary human hepatocytes and in the hepatocyte cell line Huh7.5.[17] Not surprisingly, HCV replicated at much lower levels in endothelial cells than in hepatocytes. Definitive confirmation of viral replication in endothelial cells was provided by using specific HCV protease inhibitors, which decreased the amount of HCV RNA in these cells. Micro-RNA (miR)-122 is hepatocyte specific and its fixation at 2 sites in the 5′ untranslated region of the virus genome is required for efficient HCV replication. As expected, miR-122 was not detected in endothelial cell lines. The transfection of these cells to express functionally active miR-122 RNA duplexes failed to promote HCV replication, demonstrating that replication is miR-122 independent in endothelial cells. Indirectly, this result also suggested that liver-specific factors are required for the action of miR-122 on HCV replication in hepatocytes.

Another important group of findings in this paper was the observation of functional consequences of HCV infection of endothelial cells. Indeed, HCV increased endothelial cell permeability and this effect was reversed when replication was inhibited by antiviral molecules. Neutralization of HCV infection with pooled anti-HCV immunoglobulins also restored endothelial cell permeability, demonstrating the direct effect of HCV on this parameter. Furthermore, the authors noted that HCV-infected endothelial cell lines expressing nonstructural protein NS5A were TUNEL positive, suggesting an effect of infection on brain endothelial cell apoptosis. Altogether, these findings suggest that the infection of endothelial cells by HCV and HCV replication in these cells in vivo are highly plausible.

These findings raise the important question of the impact of HCV replication in BBB endothelial cells on neurocognitive and psychological symptoms frequently reported by subjects infected by this virus. This issue has been evaluated in case-control and in longitudinal studies assessing patients before and after a sustained virologic response to antiviral therapy. In these works, neurologic involvement was evaluated by means of neurocognitive tests combined with quality-of-life questionnaires or by measuring magnetic resonance spectroscopy (MRS) signals. Such case-control studies raise the issue of matching patients with HCV infection who know their status and have past or present addictive behaviors and HCV-negative controls. Despite these limitations, most studies reported more fatigue, more depression, and less effectiveness in HCV-infected patients than in controls. The longitudinal approach also has drawbacks. Subjects know the results of therapy and have experienced the adverse effects of antiviral drugs for many months. Although numerous studies have reported an improvement of the quality of life after achieving a sustained virologic response,[18] only a few recent papers investigated changes in neurocognitive functions and MRS in relation to the response to therapy. The results of these studies are conflicting. In 1 study, the authors concluded that HCV eradication had a beneficial effect on cerebral metabolism and selective aspects of neurocognitive functions, especially in patients with mild disease.[19] In contrast, another comparable study concluded that HCV had a measurable effect on brain integrity in patients screened for other medical and/or psychiatric comorbidities, but that these abnormalities did not improve after viral eradication.[20] Both studies were based on a very small number of patients and the evaluation of neurocognitive functions and MRS were performed early after viral clearance. The second study[20] questioned the reversibility of brain involvement once HCV infection is cured. Indeed, because brain cells have a very low rate of regeneration, the effect of

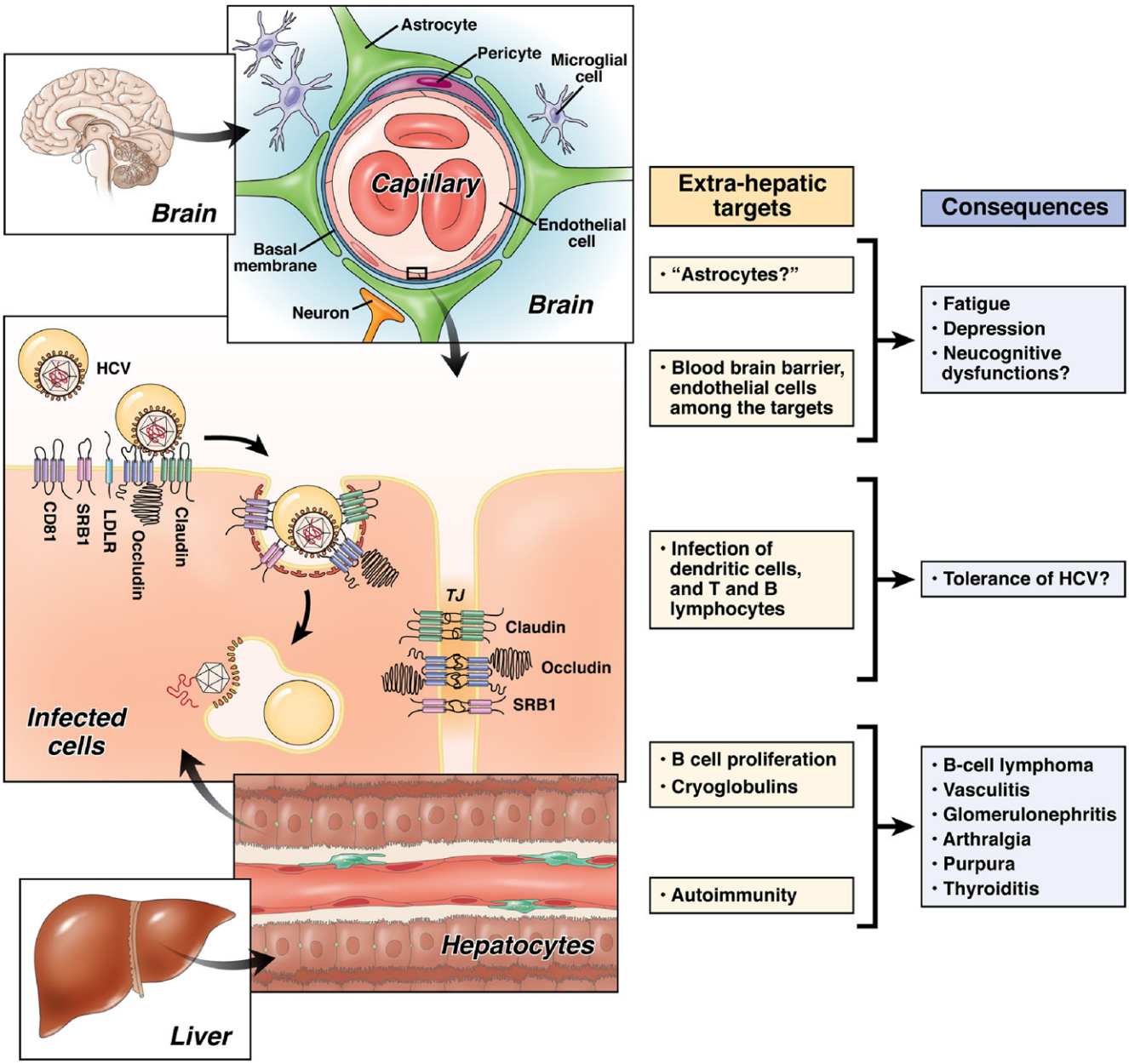

Figure 1. All the receptors of HCV exist both on hepatocytes and on the BBB endothelial cells. Extrahepatic manifestations of HCV could be related in part to interactions between HCV and extrahepatic sites of infection.

HCV infection could be prolonged by months or years after the virus has been cured.

The main interest of the article by Fletcher et al[9] is to show that the brain is a likely target for HCV. More important, the results with BBB endothelial cells suggest that other endothelial cells could be targeted and altered by HCV. For example, a study in an Egyptian population showed higher carotid intimal media thickness in HCV-infected than in noninfected patients.[21] If this is confirmed, the possibility that HCV infection could induce brain or vascular disorders could modify the current indications for therapy, which are essentially based on the severity of liver disease or the presence of extrahepatic manifestations of immune origin, such as symptomatic cryoglobulinemia or B-cell lymphoma. Further studies are thus needed in the field of HCV neuroinvasion to better define the actual consequences of infection and their reversibility if infection is eradicated. However, it must be emphasized that the effects of HCV on neurocognitive functions, depression, or fatigue are generally mild. Many HCV-infected patients are highly successful and creative. Because stigmatization of HCV infected people exists, the notion of a brain involvement in these patients could have devastating consequences. The results of the present study should therefore be interpreted as what they are, cer-

tainly not overinterpreted as the demonstration that HCV infection leads to severe brain disease (Figure 1).

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