Extra-hepatic replication of the hepatitis C virus: current issues and future directions

“...there is ample clinical relevance to efforts to define the existence and roles of extra-hepatic HCV replication sites.”

Jonathan J Hubbard¹ & Shyam Kottitil^{1*}

¹Immunopathogenesis Section, Laboratory of Immunoregulation, National Institute of Allergy & Infectious Diseases, National Institutes of Health, Department of Health & Human Sciences, Bldg 10, Room 11N204, 9000 Rockville Pike, Bethesda, MD 20892, USA

*Author for correspondence: Tel.: +1 301 435 0936 ■ skottitil@niaid.nih.gov

The hepatitis C virus (HCV) infects approximately 200 million people worldwide and 85% of infected persons develop chronic infection, which dramatically increases the risk of cirrhosis and other long-term complications [1]. Many of these long-term complications, surprisingly, involve nonhepatic tissues, including peripheral vascular tissue, renal glomeruli, peripheral neurons and lymphocytes [2]. The development of these extra-hepatic disorders, many of which seem to be associated with chronic HCV infection (CHC), could suggest that HCV can cause pathology in nonhepatic tissues [2–4] and that these extra-hepatic tissues could provide reservoirs or sites of hepatocyte-independent HCV replication. Given that overall sustained response rates to combination therapy for HCV (i.e., PEG-IFN- α plus ribavirin) are still approximately 50% [5] and that HCV almost always recurs in HCV⁺ liver transplant patients [6], there is ample clinical relevance to efforts to define the existence and roles of extra-hepatic HCV replication sites. Importantly, such efforts could lead to the discovery of ways to eradicate any extra-hepatic reservoirs of HCV, thereby decreasing the incidence of nonresponse to treatment and viral relapse following apparent sustained virological response (SVR) to therapy or liver transplantation. Currently, the questions central to confirming the occurrence and clinical relevance of extra-hepatic HCV replication include:

- Defining/confirming the role of HCV in extra-hepatic manifestations of CHC;
- Defining the extra-hepatic tropism(s) of HCV;
- Delineating the mechanisms and clinical relevance of extra-hepatic HCV replication;

- The discovering and validating of new *in vitro* and *in vivo* models of acute and CHC to facilitate more economical and expeditious delineation of the above issues.

While some progress has been made in these areas in recent years, many questions remain unresolved and future work should address these important issues. Resolution of these issues could facilitate improved clinical outcomes in HCV infection.

Role of HCV in extra-hepatic manifestations

The controversy surrounding the role of HCV in the extra-hepatic disorders/diseases previously associated with CHC is mostly related to the validity of the original clinical association studies [7]. The relevance of these questions concerning the existence of extra-hepatic HCV reservoirs/replication sites centers on the hypothesis that the increased incidence of these extra-hepatic disorders results from HCV replication in those tissues [2]. Of the sites that are likely to be important in extra-hepatic replication, lymphocytes are most consistently supported in the literature. For example, most extra-hepatic complications associated with HCV involve autoimmune disorders involving pathogenic antibodies [7], possibly suggesting chronic lymphocyte stimulation [8]. However, a direct oncogenic role of HCV in B-cell malignancies such as non-Hodgkins lymphoma [9–12], however, is unclear owing to inconsistent results between studies [7]. By contrast, the production of pathogenic antibodies by B cells in CHC, leading to mixed cryoglobulinemia (MC) and resulting in cutaneous vasculitis, is more well established [8,13,14], although systemic

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vasculitis can occur in CHC due to noncryo-precipitating immune complexes as well [15]. Studies of vasculitis in CHC have shown that vasculitis severity actually correlates with viral load and not serum cryoglobulin levels [2] and that successful HCV treatment (i.e., SVR) leads to resolution of vasculitis [13,16–18]. This observation is the most compelling clinical suggestion for a direct role of HCV in lymphoproliferative disorders and MC vasculitis. If MC vasculitis in HCV patients is a primary autoimmune disease independent of HCV infection, attaining SVR would not be expected to resolve the vasculitis. This hypothesis needs further confirmation; however, since at least one study reported a relapse of MC vasculitis in patients who had achieved SVR without finding plus- or minus-stranded HCV RNA in either cryo-precipitated immune complexes or peripheral blood mononuclear cells (PBMCs) [19].

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The role of HCV in nonlymphocytic, extra-hepatic disease is less well defined. Recent evidence suggests that HCV can replicate in the human brain [20], possibly leading to complaints of fatigue and cognitive dysfunction in some CHC patients. However, peripheral neuropathies, in CHC patients appear related to age/duration of CHC and not serum levels of cryoglobulins [21]. Associations between membranoproliferative glomerulonephritis and CHC [22–24] appear to be immune-complex-mediated and not directly due to HCV replication in the nephron, again pointing to lymphocyte involvement [7]. Similarly, the high incidence of arthralgias in CHC patients appears to be nearly universally related to antibody complex disease [25], including mixed cryoglobulins and/or rheumatoid factor [4]. Both of these observations appear to suggest at least an indirect role for HCV in the rheumatological manifestations reported in CHC patients.

Extra-hepatic HCV tropisms, mechanisms & clinical relevance

Hepatocytes clearly represent the most important site of HCV replication. However, while there is evidence supporting the hypothesis that HCV can and does replicate in B cells [26–29], at

least one review [7] observed that B-cell HCV replication occurred in abnormal B-cell environments only and suggested that these data did not, therefore, unequivocally demonstrate the existence of B-cell HCV replication. However, the demonstration of compartment-specific evolution of HCV quasispecies in lymphocytes in monoinfected and HIV co-infected subjects [28,30] could suggest that infected lymphocytes simultaneously exert selective pressure on viral replication and are themselves altered by HCV, resulting in a lymphocyte-optimized quasispecies. Therefore, given the substantial body of evidence demonstrating that CHC patients develop lymphoproliferative disorders at a high rate [13,31,32], it seems reasonable to hypothesize that HCV infection may sufficiently alter B-cell physiology to allow HCV replication to occur in B cells. Indeed, more recent data show a correlation between HCV RNA in lymphocytes and the presence of at least one lymphoproliferation marker [33]. HCV has also demonstrated low-level replication in and tropism for lymphocytes, specifically non-T-cell species of monocytes, natural killer cells and B cells, in HCV-infected patients co-infected HIV, where immune dysregulation is a hallmark [34]. In addition to these human data, *in vitro* studies have demonstrated hepatocyte-independent HCV replication in lymphoblastoid cells [28]. Additional support for lymphocyte replication comes from nonhuman primate studies, which show nonrandom evolution of unique HCV sequences in hepatic and nonhepatic tissues [29]. In addition, a study in HCV mono-infected patients found that HCV could be detected in serum and PBMCs up to 60 months after demonstrating SVR [35], suggesting that PBMCs could represent a dormant reservoir for HCV in some SVR patients, although this observation needs confirmation. Another study found that the presence of negative-stranded HCV RNA on/in PBMCs correlates with decreased rates of SVR following combination therapy [36].

One additional piece of clinical data regarding patients achieving SVR prompts intriguing questions about the existence of an extra-hepatic reservoir. Specifically, the rare occurrence of late relapses in patients previously demonstrating SVR prompts the question: was the virus passed from some PBMCs to hepatocytes? For example, one study has shown that, while most SVR patients remain disease free long term, some eventually relapse [37]. Overall, clinical studies demonstrate contradictory findings regarding the persistence of HCV in PBMCs and/or

hepatocytes of SVR patients [35,38–43]. Whether these contradictions result from differences in methods or assay sensitivities is unclear.

Future studies should address whether PBMC/lymphocyte-harbored HCV are associated with relapse following SVR, the mechanisms of HCV replication in lymphocytes and whether/how standard combination HCV therapy affects HCV in lymphocytes. Confirming or ruling out PBMCs as reservoirs for HCV in SVR or nonresponse to treatment outcomes would greatly facilitate the definition of extra-hepatic PBMC-based HCV replication in CHC complications and relapse following successful treatment. The emergence of new, more specific drugs against HCV in the near future adds another intriguing context to these questions [44]: the fact that these new treatments have so far demonstrated efficacy only in addition to the standard combination treatment [45,46] questions whether these new treatments attack HCV in nonhepatocyte compartments. The current literature does not describe the pharmacokinetics or pharmacodynamics of new agents within PBMCs or other potential extra-hepatic compartments. Demonstrating the effectiveness of these new therapies in these compartments would represent an important addition to the current understanding of their potential efficacy in relapsed CHC. Fortunately, the last decade or so has brought significant advances in laboratory models for studying HCV (reviewed in [47]) and many of these questions will hopefully be answered soon.

The future of HCV research is challenging and the quest to discover the relevance and mechanisms of extra-hepatic HCV tropisms and replication represents an important area of focus for the field. First, clinical associations between extra-hepatic manifestations of CHC and HCV need to be strengthened by well-designed case-control studies. More robust clinical data could then potentially clarify areas of highest impact for extra-hepatic HCV research. Future *in vitro* mechanistic work regarding extra-hepatic HCV processes should progress in the context of more robust clinical data and should seek to define which cells harbor and propagate HCV and how these processes affect clinical care/outcomes in CHC patients. As these goals are met, investigations into methods of treating/eradicating extra-hepatic reservoirs of HCV could provide important advances in HCV therapy, especially in CHC, and could improve the morbidity and mortality associated with HCV and CHC.

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