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REVIEW

Hepatitis C virus and peripheral blood mononuclear cell reservoirs

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Abstract

The existence of hepatitis C virus (HCV) infection in extrahepatic sites has been widely demonstrated. Since peripheral blood mononuclear cells have been the most investigated, compelling evidence of an association with HCV has been shown. Different studies have revealed that HCV RNA can persist and replicate in immune cells but the relevance of its presence and persistence over time is still unknown. As the contribution of this extrahepatic reservoir could have several clinical implications in viral transmission, treatment response and disease pathogenesis, future studies are required to improve our knowledge of the extrahepatic manifestations of HCV and its possible consequences.

Key words: Hepatitis C virus; Peripheral blood mononuclear cell; Reservoirs; Lymphotropism; Replication

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INTRODUCTION

Hepatitis C virus (HCV) is a small positive-strand RNA virus responsible for an important burden of chronic hepatitis and hepatic related diseases around the world[1]. Although HCV is mainly hepatotropic, its presence in extrahepatic sites has been widely demonstrated[2] and it was calculated that the contribution of this second compartment is responsible for about 3.1% of virus in circulation[3]. Lymphoid cells are the most investigated extrahepatic site.

HCV infection of lymphoid cells was suggested for the first time by Hellings in 1985[4]. Mononuclear leucocytes (mainly lymphocytes), isolated by Ficoll-Paque gradient centrifugation of blood freshly drawn from a hemophilia A patient with chronic non-A, non-B hepatitis (NANB), caused NANB when infused in a susceptible chimpanzee.

Immediately after discovery of the virus in 1989[5], different groups attempted to demonstrate HCV replication in lymphoid cells by infecting macrophages, B and T lymphocytes[6-9]. Moreover, several reports describing the presence of the replicative intermediate or negative strand in peripheral blood mononuclear cells (PBMC) were published[9,10]. The HCV RNA negative strand is a viral replicative intermediate and its presence can be considered direct evidence of ongoing viral replication. Nevertheless, discordant results were obtained by different groups, and the association of HCV with PBMC and viral replication in this extrahepatic site remained controversial for many years[11-16]. Strong evidence for *in vivo* HCV infection of, and replication in PBMC was provided by Bronowicki and collaborators[17]. They demonstrated the persistence of the viral RNA sequences in mononuclear blood cells inoculated into immunosup-

pressed mice and they were able to perform a second *in vivo* passage by successful transmission of HCV-RNA-positive cells to other mice. However, later on, the SCID mice did not offer a suitable *in vivo* model to study HCV pathogenesis. Different studies have reported evidence for HCV replication in granulocytes, monocytes/macrophages, dendritic cells, B and T lymphocytes[18-23]. In addition, successful infections of lymphoid cells or establishment of stable HCV+ cell lines have been achieved[22,24].

HCV REPLICATION IN PBMC

As discussed above, the detection of replicative forms of HCV RNA in PBMC has been extensively reported but remains controversial. Earlier PCR methods have been suspected to lack specificity and/or sensitivity, possibly due to the very low concentration of negative HCV RNA strand in cells. Currently, methodological modifications have been used to overcome these difficulties and many reports demonstrated that HCV can certainly replicate in PBMC[25-29].

Some studies showed that replication in PBMC occurs at a very low level and the amount of intracellular HCV RNA is patient-specific and is a result of a dynamic process related to virologic and immunologic factors[26,28]. The role of HCV lymphotropism in the natural history of HCV infection is not yet resolved and reports remain arguable. Nowadays, although it is accepted that HCV can replicate in PBMC, the contribution of this extrahepatic site as a significant viral reservoir and the importance of viral persistence in aviremic subjects after spontaneous or therapeutical clearance is still under debate.

PBMC AS HCV RESERVOIRS

Lymphoid cells may represent privileged reservoirs that could favor HCV persistence leading to chronic HCV infection. The infection of immune cells may interfere with the efficiency of viral clearance by the host[30,31]. Different reports demonstrate the persistence of HCV RNA at very low levels in serum and peripheral lymphoid cells after apparently complete spontaneous or antiviral therapy-induced resolution of chronic hepatitis C[32,33]. The occult HCV persistence in lymphoid cells may have important epidemiological and pathogenic implications. Radkowski and collaborators[33] suggested that in patients with sustained virological response (SVR), small quantities of HCV RNA may persist in liver or PBMC for up to 9 years. These findings could explain the phenomenon of frequent persistence of humoral and cellular immunity for many years after supposed viral clearance but also, could present a potential risk for transmission and reactivation. It was also demonstrated that HCV may also persist and replicate in the liver and PBMC of healthy, anti-HCV antibody-positive, serum HCV RNA-negative patients who have persistently normal ALT levels[34]. It is possible that viral persistence and, specifically, the presence of HCV RNA in PBMC may lead to HCV reactivation under special circumstances. In patients with immunosuppresion, under immunomodulatory therapy or with coinfection, persistent replicating HCV could represent a potential source for viral recurrence. These findings suggest that sterilizing immunity with complete elimination of virus is unlikely.

EVIDENCE AGAINST PBMC AS LONG-LIVED HCV RESERVOIRS

In contrast with the above mentioned reports, some studies refute the role of PBMC as a long-lived HCV reservoir. Kaiser P and collaborators[26] evaluated 30 HIV/HCV coinfected patients for up to 40 mo. Total PBMC-associated HCV RNA and virion-enclosed PBMC-associated HCV RNA, that could represent viral particles nonspecifically attached to blood cells, were distinguished and they observed widespread presence of viral RNA in PBMC from HCV-viremic patients. Evidence for persistence of HCV in PBMC in the absence of HCV viremia in plasma could not be found. Their experiments supported a concept of low level replication in PBMC and suggested that the infection and expression of HCV in PBMC is of minor quantitative importance for systemic replication and persistence of HCV[26].

Another report in which HCV persistence was underestimated was published by Bernardin[35]. In their experiments, they could not find any HCV RNA detectable PBMC sample in 69 aviremic donors indicating that PBMC are unlikely to serve as a long-lived reservoir of HCV in aviremic subjects.

The slow decrease in anti-HCV antibody titers in subjects with spontaneously cleared viremia as well as the complete seroreversion detected in 7% of transfusion transmitted infections may also reflect an absence of ongoing antigenic stimulation, indirectly supporting clearance of infection in persons who test HCV RNA-negative in plasma[35].

HCV LYMPHOTROPISM

The presence of variants of the highly conserved 5' untranslated region (UTR) have been observed between HCV from plasma and PBMC[36,37]. The identification of sequence polymorphisms in cells of the lymphatic system suggested possible adaptation of HCV to replicate in nonhepatic cells[29]. In addition, a compartmental distribution of HCV quasispecies and HCV genotypes has been demonstrated[37,38]. Concordant with our results in a hemophilic population, the HCV genotypes detected in PBMC were not detected in plasma in some individuals supporting independent replication in these cells[37,39].

These findings further support the lymphotropic nature of HCV and reinforce the concept that independent replication of HCV in lymphoid cells may constitute a potential risk for persistence, reactivation, recurrence or treatment resistance.

CLINICAL IMPLICATIONS OF PBMC AS HCV RESERVOIRS

HCV transmission

In some contexts, as in vertical transmission, the presence of HCV in PBMC played relevant roles. The risk of mother-to-child transmission is associated with the presence of HCV in maternal PBMC[40]. Likewise, the persistence of small quantities of HCV RNA in aviremic patients who are supposed to have solved the infection could have important implications for viral transmission.

Reactivation or recurrence

Several studies demonstrate that relapse after sustained virological response is extremely rare[41]. However, low-level intrahepatic viraemia despite negative serum HCV RNA testing has been shown to predict a higher likelihood of late relapse, particularly in the setting of immunosuppression[41].

The existence of PBMC reservoirs may be implicated in the recurrence of chronic hepatitis after apparently successful antiviral treatment. Previous findings suggest that in patients with spontaneous eradication or sustained virological response after therapy, small quantities of HCV RNA may persist in lymphoid cells for years[32,33]. The presence of positive/negative strand HCV RNA at the end of treatment was associated with relapse among HCV-HIV coinfected patients[42]. It has been suggested that low level replication of HCV in PBMCs may lead to reactivation of HCV after termination of therapy[2,32,33].

Reemergence of HCV RNA was demonstrated in apparent sustained viral responders receiving immune suppressive therapy[43,44]. This proved that the HCV reservoir requires continued innate or T cell immune surveillance to prevent disease activity even years after the infection in at least some sustained viral responders[43].

On the other hand, recurrent infection in transplant recipients was also described[45-47] and the utilization of antiviral therapy in HCV-infected patients awaiting liver transplantation as one of the strategies to prevent hepatitis C recurrence after transplantation was recommended[48]. It has been proposed that viral variants from extrahepatic compartments may be involved in infection recurrence after liver transplantation. One report has specifically investigated the origin of HCV recurrence and suggested that liver-derived virus remaining in circulation was the major responsible for the graft reinfection. However, virus variants of likely extrahepatic origin could be detected in serum early after transplantation[45].

Immune dysfunction and lymphoproliferative disorders

The interaction between HCV and the human immune system is likely to have important clinical consequences. First, HCV has a remarkable ability to evade the immune system, achieving almost 85% chronicity rates. On the other hand, HCV infection may induce extra-hepatic immune related manifestations in a high percentage of infected patients, including mixed cryoglobulinemia and non-Hodgkin lymphoma. At present, the possible mechanisms by which HCV modulates immune function are being examined.

Monocytes, B cells, and CD4+ and CD8+ lymphocytes can support HCV replication and can serve as reservoirs in symptomatic and occult HCV infections[29]. Endogenous presentation of HCV antigens by infected B cells and monocytes may contribute to immune tolerance of HCV, favoring its persistence[29]. Particularly, in perihepatic lymph nodes, HCV replication has been demonstrated and the results suggest that replication of HCV in T cells might contribute to disturbance of Th1 commitment or Th1 hyporesponsiveness in individuals with persistent HCV infection[31].

According to some reports, a predominant infection of B lymphocytes suggests a preferential tropism of HCV for B cells[49,24]. In consequence, chronic antigen stimulation by the virus may trigger B cell proliferation resulting in a wide spectrum of lymphoproliferative disorders, cryoglobulinemia and non-Hodgkin lymphoma, frequently observed in infected patients[2,50].

Treatment resistance

Different factors have been associated with lower treatment response, such as higher serum HCV viral load at baseline, HCV genotype 1 infection, co-treatment with antiretroviral therapy, the presence of IFN-neutralizing antibodies and a higher degree of immune deterioration[51-56]. The detection of HCV RNA in PBMC reservoirs might have important implications for effective treatment. One possible mechanism of relapse is that PBMC could serve as a viral reservoir resistant to IFN[30]. As demonstrated, clearance of HCV RNA in PBMC at the end of IFN treatment was a predictor of durable response to antiviral therapy in patients with chronic hepatitis[57]. Moreover, in the context of HCV-HIV coinfected patients, the presence of strand specific HCV RNA at the end of 48 wk of therapy was associated with viral relapse[41].

On the other hand, HCV lymphotropic variants corresponding to genotypes more resistant to treatment could persist in lymphoid cells[58,39]. Interestingly, Di Liberto and collaborators found that the presence of compartmentalization in PBMC was strongly predictive of sustained virologic response[59].

CONCLUSION

To date, different reports suggest that HCV replication in PBMC does definitely occur which proved to have important effects on different aspects of HCV infection. Nonetheless, as conflicting findings *in vivo* still exist, the contribution of this extrahepatic site in disease pathogenesis and treatment should be further explored and the mechanisms involved should be elucidated.

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