

Editorial

## The anhepatic phase of liver transplantation as a model for measuring the extra-hepatic replication of hepatitis C virus

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In spite of a large body of literature devoted to the replication of HCV in other cells than hepatocytes, the reality and a fortiori the consequences of such extra-hepatic replication remain debated and elusive.

In the absence of specific, sensitive and reliable immunohistochemical assays [1], the essential tool to assess the possible replication of HCV in non-hepatocyte cells is the quantitative and qualitative analysis of HCV RNA. The detection of the positively stranded HCV RNA in an extra-hepatic tissue is clearly insufficient to demonstrate the viral replication in this tissue, the absorption of circulating viral particles containing the viral genome being difficult to exclude. However, it is conceivable that the binding of viral particles to extra-hepatic cells without replication could have some pathological consequences [2]. Two main approaches based on the analysis of viral nucleic acids have been used to assess the replication of HCV in extra hepatic sites. The first one is the detection of the HCV RNA negative strand, an obligatory replicative intermediate that is not found in plasma [3]. Its presence theoretically demonstrates that at least the first phase of the replication exists meaning that the viral polymerase is active and thus that the viral polyprotein has been translated then matured. The detection of HCV RNA negative strand is based on its reverse transcription by using a sense (e.g. positive) primer followed by PCR. However, inter- and intra-priming of viral nucleic acids render possible the reverse transcription of HCV RNA positive strand even without the appropriate primer leading to false positive results. In some recent papers, concentrations of more than  $10^6$  HCV RNA positive strand/ml frequently lead to a false positive detection of HCV RNA negative strand. Thus, the accurate detection of

negatively stranded HCV RNA can only be performed in samples containing a low concentration of positively stranded HCV RNA. Since the fraction of negative vs. positive strands is generally estimated to be less than 10%, the detection of HCV RNA negative strand is rather difficult. The detection and in some cases the quantification of negatively-stranded HCV RNA has been performed in peripheral blood mononuclear cells (PBMC), B lymphocytes, monocytes/dendritic cells, total peripheral blood mononuclear cells (PBMC) by many authors [3,4]. Its detection is almost universal when tested in the liver but not in extra-hepatic tissues indicating a likely low level of replication, which is suggested by Dahari's paper [5]. More generally, most studies suggest a significantly lower level of replication (positive and negative strands), in lymphocytes or monocytes than in the liver compartment.

The second approach used by the others to assess the putative replication of HCV in extra hepatic tissues or cells is based on the comparison of variants or quasispecies detected in different compartments: plasma, liver and PBMC for instance. The non-random distribution of these quasispecies, described as the compartmentalization of quasispecies [6] strongly supports the replication of HCV in PBMC. We demonstrated through the analysis of the hypervariable region 1 of HCV that this phenomenon was frequent, likely constant and mainly involved B cells, monocytes, in some cases CD8+ T lymphocytes and rarely CD4+ lymphocyte [7]. A major observation pointing to an autonomous extra-hepatic replication of HCV is the infection of an extra-hepatic site by a genotype not found in the plasma or the liver [4,8]. Given the large phylogenetic distances between different HCV genotypes, the possibility that a genotype found only in PBMC had derived from the genotype found in plasma can be excluded. On a large series of 113 patients, we recently identified more frequently in liver transplanted patients, multitransfused subjects and

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former drug users, a high prevalence (around 25%) of coinfection by two genotypes, one being present only in PBMC and not detectable through cloning or Line probe Assay in plasma [9] and the other being predominant in the plasma. In the same paper, we demonstrated that this compartmentalization could persist during years and even after liver transplantation and that plasma and liver HCV genotypes were identical. These observations are in accordance with Dahari's results suggesting that extra-hepatic sites produce only a small proportion of plasma virions. In the setting of liver transplantation, Laskus et al. [10] showed that PBMC variants could be detected in plasma during and few days after the anhepatic phase but that finally plasma variants found before transplantation became predominant in plasma shortly thereafter. All these findings point to a low replication of HCV in non-hepatocytic cells and therefore to a low contribution of the extra-hepatic sites to the plasma viral load.

Dahari's elegant study uses patients undergoing orthotopic total liver transplantation and, more precisely during its obligatory anhepatic phase as a quasi-experimental model for analyzing the kinetics of HCV RNA during the rapid and transitory removal of the major replicative compartment. Three models were applied and compared with the observed measurements of HCV viremia. The first model is a single compartment model in which only one replicative compartment exists and is the source of plasmatic virions. The second model integrates two replicative compartments both contributing to the plasma viral load. The third model is based on the assumption that HCV is bound to an extra-hepatic compartment but does not replicate within it (binding compartment model) and is released into the circulation with a slow rate. The interesting point was that the two-compartments model was found to be the best correlate with viral kinetics during and after the anhepatic phase.

Let us suppose that no replication of HCV exists in extra-hepatic sites. What could be the other possibility mimicking the two-compartments model? In Dahari's paper as well as in most models of viral kinetics, the rates of production, binding and destruction of HCV RNA according to a given compartment are supposed to be identical for all viral particles and constant over time. This assumption is not obvious. The quasispecies composition of HCV RNA detected in plasma as well as in the different replicative compartments is usually heterogeneous and changes over time. One may hypothesize that in plasma and at a given time, two or more viral populations having different life spans coexist. The anhepatic phase could lead to a rapid decline of viral particles with the shorter life spans followed by the slower decline of remaining viral particles with the longer life spans. This is also a possibility for kinetic studies of viral load during antiviral therapy [11]. The heterogeneity of HCV in plasma also exists at a physical degree with the well-established heterogeneity of HCV particles shown by density gradient analysis [12]. An interesting development should be to

simultaneously perform a kinetic analysis of the viral load together with the analysis of the genetic or physical heterogeneity of HCV. In this view, a phylogenetic clustering could exist between quasispecies found during the second phase (of the two-compartments model) and quasispecies found in an extra-hepatic site (for instance lymphocytes or monocytes); the kinetics and thus the life spans of low- and high-density HCV particles in the setting of the anhepatic phase should be interesting to determine.

Taken together all data available to date, the extra-hepatic replication or propagation of HCV in non-hepatocytic cells is more than probable. What are the consequences of such extra-hepatic replication? An optimistic hypothesis may be that strains or quasispecies adapted to non-hepatocytic cells are unfit to replicate in hepatocytes and do not infect them. This is supported by the finding of HCV RNA in PBMC of long-term responders to interferon-alpha [13,14]. This could explain the persistence of anti-HCV antibodies in patients with sustained virological response after interferon-alpha although memory B cells may also be maintained by polyclonal stimuli [15]. Another possibility should be that the replication of HCV in some extra-hepatic sites (for instance immune cells) could modulate the immune response mounted against the variants or quasispecies infecting the hepatocytes and finally could favor the persistence of liver infection. A direct pathogenicity of HCV in lymphocytes or monocytes is suggested by the previously reported link between lymphomagenesis and HCV and the functional abnormalities of dendritic cells infected by HCV. In conclusion, the production of HCV virions by extra-hepatic sites is likely low but the infection of such extra-hepatic sites may play a major role in the persistence of HCV.

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