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Letter to the Editor

Trying to catch the HCV virus in its 'battle field'

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Dear Editor,

The hepatitis C virus (HCV) was identified in 1989[1] and is known to be one major causative agent of chronic liver diseases. Unlike other forms of viral hepatitis, hepatitis C infection is very difficult for the immune system to overcome; consequently in most cases hepatitis C becomes chronic, resulting in a wide clinical spectrum of liver failure leading to cirrhosis in about 20% of infected patients, and with an increased risk of developing hepatocellular carcinoma.

The viral genome is a single stranded, positive-sense RNA that exhibits a considerable degree of sequence variation. Based on this variation, HCV can be classified into at least six major genotypes, which can be further divided into a total of more than 40 subtypes. HCV infection is associated with hepatocellular injury, with massive necrosis and a dramatic increase of AST release, accompanied by different degrees of hepatic inflammation and fibrosis. Differences in genotype do not seem to correlated with disease progression, even though specific genotypes have been shown to be associated with distinct histopathological manifestations.[2] The virus can multiply in lymphoid cells, but the primary targets of HCV are hepatocytes. In chronically infected liver, the viral replication rate is extremely high and the number of infected hepatocytes is estimated to be 50% or more.[3] However the mechanism of progression of disease is yet not known. Different factors appear to be involved, in particular the host immune reaction that, although inefficient at containing viral infection, is sufficient to cause collateral liver injury, due to infiltrating lymphocytes within the hepatic parenchyma and the production of inflammatory cytokines.[4] Hepatic steatosis is another frequent feature of chronic hepatitis C, and its presence accelerates progression of fibrosis. Some reports indicate that HCV, through its core protein, may directly cause lipid accumulation in hepatocytes, altering mitochondria membrane structure and causing impairment of lipid oxidation.[5,6]

Unfortunately, to date knowledge remains limited on the ultrastructural modifications occurring in hepatocytes that accompany the development of disease. In fact, although the organization of the viral genome has been described in detail, the characterization of the hepatitis C agent morphogenesis is still matter of debate. Flavivirus-like particles 55–70 nm in diameter, with a 35–50 nm inner core, have been isolated from human serum and described as spherical particles with fine surface spike-like projections.[7] Despite many efforts, the search for the morphological features of HCV inside hepatocytes has been hampered probably because the synthesis of HCV proteins is extremely low in the liver, making it difficult to visualize the virus, and by the lack of a convenient immunohistochemical marker. In keeping with these problems, little information is available on intracellular localization of the virus and on the prerequisites for its assembly.

Our work has been focused on the ultrastructural modifications induced by HCV in the hepatocytes of infected human liver, in order to understand whether cell damage is related to a direct cytophatic effect of the virus on hepatocytes. To date hepatitis C virus particles and related induced morphological changes have been described in only a limited number of studies performed in cell cultures, especially with T- and B- cell lines.[8,9] In these studies it has been reported that virus particles localize inside cytoplasmic vesicles, apparently dilated cisternae of the endoplasmic reticulum. In a more recent study, performed on hepatoma cell lines transfected with HCV replicons, association of the majority of viral proteins with intracellular membranes has been reported, suggesting that newly formed virus particles acquire their envelope in the lumen of the endoplasmic reticulum, and that virus release presumably takes place via transport through the Golgi compartment.[10] Beside these results, the localization and the assembly of the virus are still controversial. Viral particles were never definitively detected in infected human liver and only rarely observed in hepatocytes of infected chimpanzees.[8] It is possible that in '*in vivo*' hepatocytes newly formed virions were rapidly released from the cell, while in cultured susceptible cells virus particles remain sequestered inside vacuoles since their release requires some host factors and thus couldn't take place. Also for the subcellular localization of viral proteins different results have been obtained according to the experimental system used; for example the HCV NS3 protein can have a nuclear localization or not depending on the presence of the NS4A cofactor,[11] suggesting once more the importance of studying HCV in its natural environment.

We have recently analyzed, by transmission electron microscopy, a number of hepatic biopsies from HCV positive patients of various gender and age, and infected by hepatitis C virus of different genotypes. Our electron microscopy observations revealed that a number of hepatocytes displayed a variety of morphological alterations, which are considered as characteristic of flavivirus infection,[12,13] and that we never detected in control livers. In particular an excessive proliferation of membrane-bound organelles mainly consisting of endoplasmic reticulum derived vesicles and accumulation of membranous structures were observed (Figure 1a). Furthermore, in some cases alteration of mitochondria was visible together with the presence of lipid inclusions in the cytoplasm.

In a limited number of hepatocytes, clearly recognizable for the peculiar features of their cytoplasm, with great

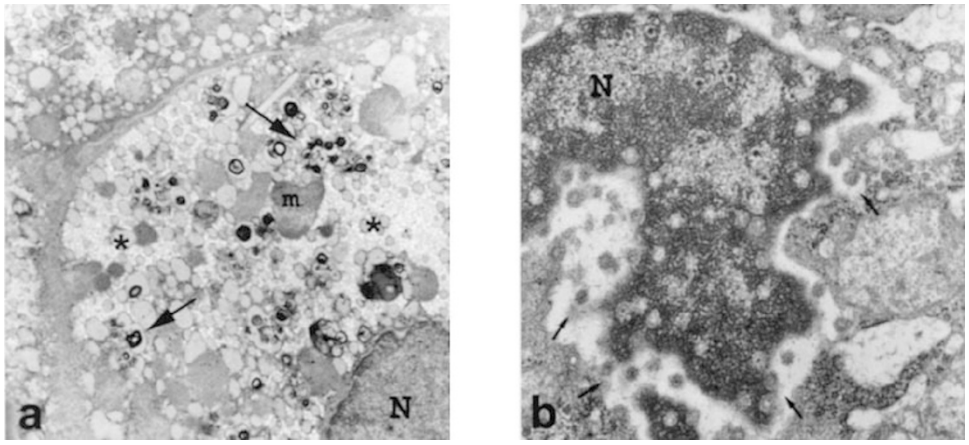

Figure 1 Transmission electron micrographs of HCV-infected human liver. (**a**) Hepatocytes display a variety of ultrastructural morphological changes, with alteration of mitochondria (m) and proliferation of endoplasmic reticulum derived vesicles (*). In addition the accumulation of peculiar membranous structures (arrows), consisting of aggregates of intracytoplasmic membranes, was found. (**b**) Hepatocyte displaying alterations of nuclear morphology typical of the early sign of apoptosis: indentation and condensation of chromatin at the periphery and detachment of the nuclear membrane. Along the perinuclear space many virus-like particles are visible (arrows). Original magnifications: (**a**) 7000×; (**b**) 20000×

abundance of endoplasmic reticulum, lysosomes and lipid inclusions, we have noticed changes of nuclear morphology. The nucleus was strongly affected, with loss of the typical round shape, indentation and partially condensed chromatin at the periphery, and detachment of the nuclear membrane (Figure 1b). These morphological alterations are characteristic of the early phase of the apoptotic process. It is still unclear whether apoptosis contributes to the pathogenesis of HCV disease (see Schulze-Olstoff this issue). Recent data demonstrated that in liver from HCV infected patients only a small number of hepatocytes undergo apoptosis, even though a greater number of cells appear primed for cell death.[14] We observed that in association with these nuclear modifications there was the presence of circular particles with an approximative diameter of 50 nm. The morphology and the size of these particles were consistent with the predicted HCV virions. As shown in Figure 1b, virus-like particles were randomly localized in the perinuclear space and sporadically seem to be budding from the nuclear membrane. Particles were never found either inside cytoplasmic vesicles or scattered throughout the cytoplasm. We have observed the virus-like particles in 80% of the liver biopsies from HCV infected patients analysed, but we never found these structures in healthy livers or livers affected by other hepatic disorders. Anyway, the number of hepatocytes displaying viral particles was always extremely low, independently from the viral load of the patient.

Taken together the data reported suggest that HCV infection is not *per se* cytopathic for hepatic cells, considering that despite the large amount of intracellular viral RNA, the production of viral progeny within hepatocytes is very low. On the other hand, it can be hypothesized that, if viral particles are formed in cells which are pre-apoptotic, these cells are rapidly cleared after death, and thus the viral particles are also degraded. This hypothesis is in agreement with studies performed in cell culture showing that the assembly of HCV virions is associated with development of cytopathic effects.

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