

Applications of confocal microscopy in the study of viral replication in hepatocytes of PCR-negative patients with Hepatitis C Virus

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ABSTRACT

It is presumed that resolution of hepatitis C, as evidenced by normalization of liver function tests and disappearance of hepatitis C virus (HCV) RNA from serum, as determined by conventional laboratory assays, reflects virus eradication (1). In this study, we examined the expression of HCV components in liver biopsies and peripheral blood mononuclear cells (PBMC) from 25 patients seropositive for HCV antigens but negative for HCV-RNA in serum (52% of these patients have an apparently complete spontaneous or antiviral therapy-induced resolution of chronic hepatitis C). Samples were analyzed by an in situ hybridization (ISH) assay using specific probes for detection of HCV-RNA of negative and positive polarity. In addition, an immunofluorescence assay using antibodies specific for HCV core, E1 and E2 antigens was performed. Apoptosis was assessed by TUNEL assay and detection of caspase 3 activation. Samples were viewed with a 60x (NA 1.4) objective on a Nikon microscope with attached laser confocal scanning system MRC 600 (BioRad, Watfod).

The HCV-RNA of positive polarity was detected in the biopsy specimens from 23 out of 25 samples analyzed (92%) (Figure 1A). The HCV-RNA of negative polarity was detected in the biopsy specimens from 21 out of 25 patients analyzed (84%), suggesting progressing virus replication (Figure 1B). No hybridization signals were observed from the negative control subjects (Figure 1C). It is interesting to note that some hepatocytes with positive signals showed a fragmented nuclei which possible represent apoptotic cells. In addition, reactions products suggestive of HCV antigens within hepatocytes were observed in all analyzed samples by immunofluorescence (Figure 1D).

ISH detected HCV-RNA of positive polarity in PBMC from 16 out of 17 samples analyzed (94%) but not in PBMC from the control subjects (Figure 1E). Besides, the HCV-RNA of negative polarity was detected in 14 out of 17 samples analyzed (82%), suggesting the presence of virus replication (Figure 1F). No positive signals were observed in PBMC from the control subjects.

The presence of apoptotic hepatocytes in the liver biopsies specimens and PBMC was analyzed using the TUNEL assay. Figure 1G shows a representative experimental demonstrating that some hepatocytes and PBMC from HCV-infected patients were undergoing apoptosis as indicated by dUTP incorporation. However, no sign of apoptosis was detected in either hepatocytes or PBMC from uninfected patients. Finally, the activation of caspase 3 was investigated to verify the detection of apoptotic hepatocytes. Almost no immunoreactivity was evident in healthy liver tissue using antibodies specific for activated caspase 3. In contrast, liver tissue from patients clearly showed hepatocytes that stained positively for active caspase 3 (Figure 1H).

These results imply that HCV RNA including intermediates of replicative form of the HCV genome can persist in the liver and PBMC after apparently complete spontaneous or antiviral therapy-induced resolution of chronic hepatitis C. In addition, data indicate the presence of apoptosis in HCV-infected hepatocytes and PBMC.

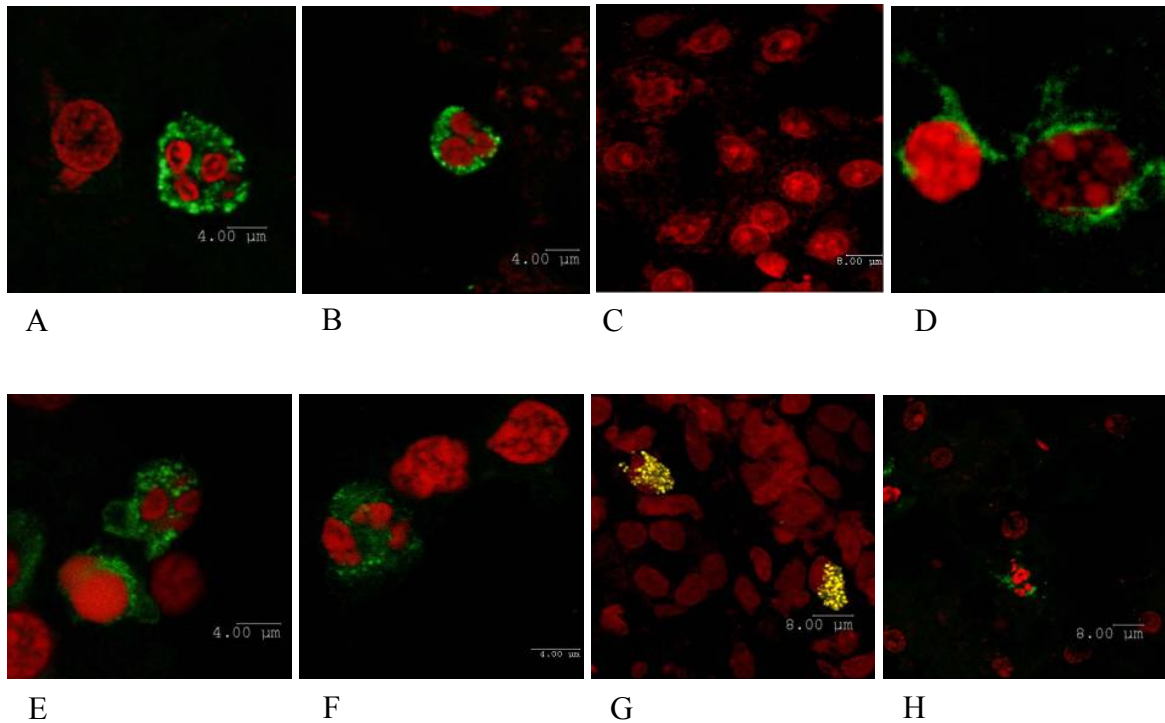


Figure 1. Detection of HCV-RNA of positive polarity (A,E) or HCV-RNA of negative polarity (B,F), or HCV core antigen in either a liver biopsy specimen (A,B,D) or PBMC (E,F) from an infected patient. Absence of hybridization signals in a liver biopsy specimen from an HCV-uninfected healthy donor liver (C). Detection of DNA fragmentation by TUNEL assay (G) and caspase 3 activation (H) in a liver biopsy from an infected patient.

References

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