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News and Commentary

Does nitric oxide play a pathogenic role in hepatitis C virus infection?

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In spite of important progress in understanding the mechanisms of hepatitis C virus (HCV) pathogenesis in the last decade, we still lack firm experimental evidence to explain the two major features of the natural history of this viral infection: viral persistence and hepatic damage. Nevertheless, recent information from both *in vitro* HCV replication systems and animal models of HCV infection have led to a plethora of critical observations showing that host immune responses play a critical role influencing the outcome of HCV-induced liver disease, such as favoring virus persistence and by mediating liver cell injury.[1] In an attempt to reconcile these apparently opposite pathogenic pathways, the increasing evidence that nitric oxide (NO) is one of the most versatile mediators in the control of viral infections, being the earliest host's antiviral response[2], as well as in the pathogenesis of many human infectious and inflammatory diseases,[3,4] acting as a pro-apoptotic inducer in some cell types or as an anti-apoptotic modulator in other cell types including hepatocytes,[5] makes it reasonable to consider this multifunctional molecule as a potential player in HCV pathogenesis. This notion has been reinforced since the observation in the liver of HCV-infected patients of an enhanced iNOS expression, implying an excessive NO formation, positively correlated with viral load and hepatic inflammation.[6–8] The ultimate effect of iNOS-derived NO on HCV pathogenesis, however, still remains far from completely understood but, in this commentary, we will try to shed light on the NO paradox in the pathogenesis of HCV infection.

The most striking feature of hepatitis C is its marked tendency toward chronicity. To this end, HCV must either overcome or not induce an effective antiviral immune response, or it must be able to evade it. Perhaps one of the simplest explanation to the enigma of HCV persistence in liver cells is that HCV replication is not affected by NO, as recently demonstrated by Frese *et al*.[9] They convincingly showed, studying the effect of both a NO donor and a specific inhibitor of iNOS on HCV replicons in cultured human hepatoma cells, that viral protein synthesis and RNA replication is resistant to NO. In addition, NO may impair antiviral responses by suppressing type 1 helper T cell responses, as recently shown in iNOS-deficient mice,[10] allowing the virus to overcome the pressure of the cellular immune system. Another important cause of viral persistence is an extensive generation of viral escape mutations, a situation frequently seen in HCV infection,[1] and evidence exists that NO could favor this scenario by accelerating the mutation rate of this RNA virus during viral infection *in vivo*.[10]

NO could also contribute to virus persistence by means of its anti-apoptotic effect in hepatocytes[5] and, likewise, HCV itself may directly influence liver cell survival by preventing apoptosis through activation of the NF-κB signaling pathway.[11] Care must be taken, however, against premature extension of these *in vitro* findings to what we can expect to find in HCV-infected individuals, but the recent observation of an increased intrahepatic NF-κB activity in these patients[12] strongly argues for an active role of this signal transduction pathway *in vivo*. Whether the close encounter of NO and HCV during the acute phase of HCV infection results in a survival advantage for HCV-infected liver cells, the persistence of the virus will probably occur thus permitting the progression to a chronic phase of HCV infection. Although this attractive theory is very difficult to test in patients, we favor this model as a possible pathogenic scenario during the acute phase of natural HCV infection.

What about the role of NO in the chronic setting of HCV infection? As mentioned before, hepatitis C leads to consistent up-regulation of hepatic iNOS gene expression and given that liver inflammation with hepatocellular damage, fibrosis and, ultimately, hepatocellular carcinoma characterize the natural history of chronic HCV infection, it is likely that NO could be an active mediator inducing liver injury and carcinogenesis. Under conditions of oxidative stress, as seen in certain chronic inflammatory disorders including hepatitis C, reactive NO species (RNOS), such as peroxynitrite and nitrogen oxides, are currently considered as the main mediators of the deleterious effects to the host of NO, including cytotoxicity and DNA damage.[3] In this regard, there is increasing experimental evidence that RNOS may play a key pathogenic role in viral diseases. In influenza A virus-infected wild-type mice, for example, an excessive production of RNOS in the lungs led to respiratory failure and death, whereas knockout mice lacking a functional iNOS gene survived the infection with little evidence of pneumonitis.[13] Moreover, in the liver of HCV-infected patients, peroxynitrite formation is markedly increased in those cases with higher inflammatory activity.[8] A direct pathogenic role of NO in chronic hepatitis C, however, is more difficult to establish, largely because of the lack of HCV-infected animal models that allow experimental suppression or modulation of NO production. Nonetheless, relevant information on the role of NO on HCV pathogenesis could be drawn from the recently developed transgenic mice expressing either the structural

proteins alone or the full complement of HCV proteins, assessing the relationship between the intrahepatic iNOS expression pattern as well as the generation of peroxynitrite and the pathological changes observed in these animals, but these studies still remain to be performed.

A major epidemiological association between chronic HCV infection and hepatocellular carcinoma (HCC) is evident but a direct pathogenic link has been recently shown in HCV transgenic mice.[14,15] However, there is an obvious inconsistency regarding the responsible viral proteins because some authors showed that the core protein alone is sufficient for tumor formation whereas others showed that the full-length HCV polyprotein is necessary to induce tumors. This discrepancy suggests the possible presence of multiple pathways in the process of HCV-induced carcinogenesis and we argue that interactions between NO and HCV proteins could explain, at least in part, these seemgly contradictory effects. In this regard, we have preliminary data that the non-structural HCV protein NS5A and, to a lesser extent, the core protein are able to induce iNOS gene expression in cultured liver cells, supporting our hypothesis that different HCV proteins and NO may interact in a synergistic manner to deliver a potent oncogenic signal to infected hepatocytes. The amounts of viral proteins expressed in liver cells along with the level of NO production in these same cells could be, therefore, a significant parameter in the pathogenesis of HCV-related HCC. However, we take a somewhat cautious viewpoint on this issue because what we actually know is that both HCV and NO have the potential to induce carcinogenesis in animal models, but not necessarily predict what they will do in natural HCV infection in humans.

What then are we to believe about the role of NO in HCV pathogenesis? It may sound glib to argue that most of these apparently unrelated effects may be real and biologically relevant *in vivo*, but the truth is that although both the immunosuppressant effect and the prevention of apoptosis mediated by NO may allow HCV to persist in liver

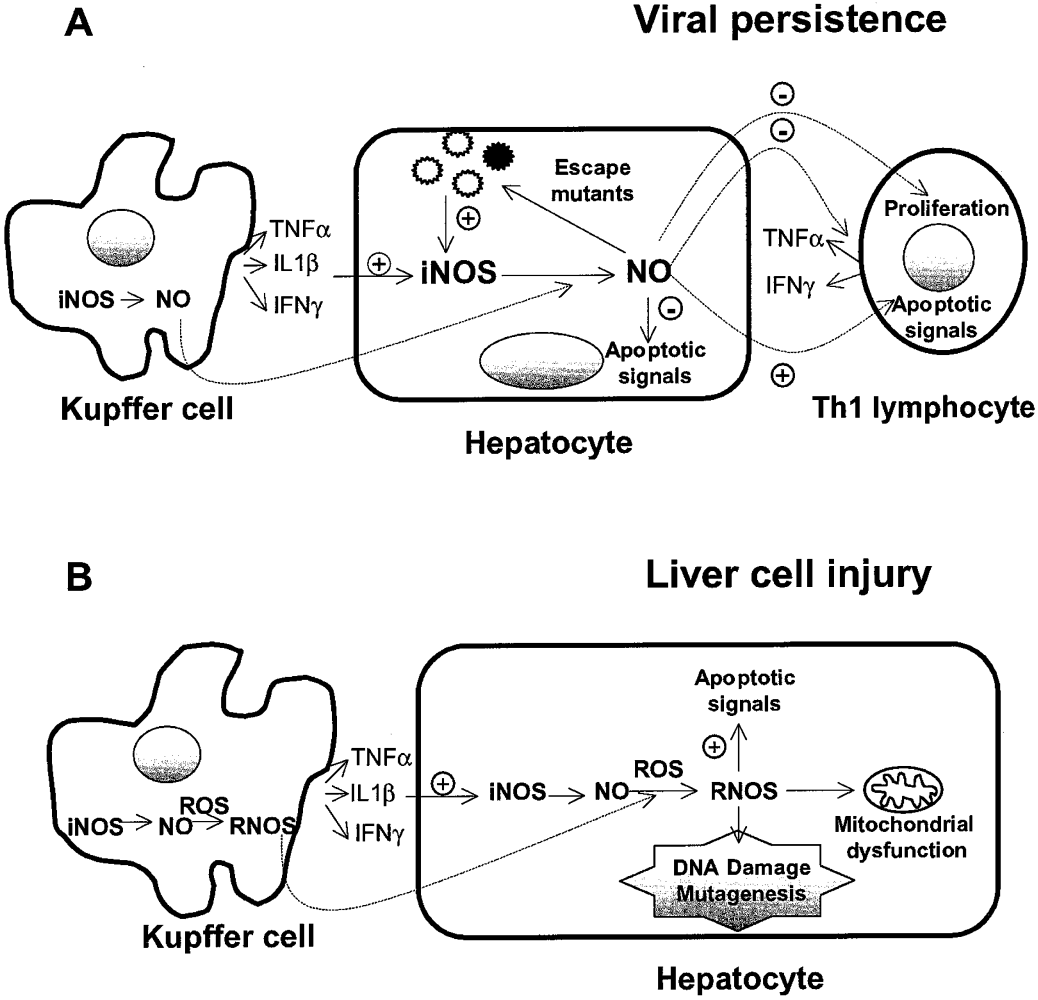

Figure 1 Proposed mechanisms by which nitric oxide (NO) could influence viral persistence in acute HCV infection are shown in (**A**). Noteworthy, NO may induce viral mutations and a selective suppressive effect on Th1 cells, thus allowing HCV to escape the host immune system. In addition, the anti-apoptotic effect of NO in hepatocytes should facilitate HCV replication in a long-lived cellular compartment. Potential mechanisms by which a persistent high NO production could contribute to liver cell injury and carcinogenesis in chronic HCV infection are shown in (**B**). The promotion of apoptosis and mitochondrial dysfunction may explain the ocurrence of liver cell injury and the induction of DNA damage could lead to mutagenesis and the subsequent development of hepatocellular carcinoma

cells and somehow contribute to carcinogenesis (see Figure 1A), the induction of hepatocellular injury and DNA damage, on the other hand, may explain the ocurrence of hepatitis and HCC, respectively (see Figure 1B). Thus, all these diverse effects of NO could be important to HCV infections. The future challenge is to elucidate how these myriad effects interact in natural HCV infections, trying to resolve the NO paradox in the pathogenesis of HCV-induced liver disease.

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