Mechanisms of Hepatic Toxicity

IV. Pathogenetic mechanisms involved in hepatitis C virus-induced liver diseases*

JOHNSON YIU-NAM LAU
Department of Antiviral Therapy, Schering-Plough Research Institute, Kenilworth, New Jersey 07033

Lau, Johnson Yiu-Nam. Mechanisms of Hepatic Toxicity. IV. Pathogenetic mechanisms involved in hepatitis C virus-induced liver diseases. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G1217–G1220, 1998.—The pathogenetic mechanisms for liver damage in acute hepatitis C are not clear, but a host immune cellular response may be involved. In chronic hepatitis C, there is strong evidence that host cellular immune response is involved in the control of viral replication and contributes to hepatocellular damage. As hepatitis C virus infection persists, continuous liver damage and regeneration, together with enhanced fibrogenesis, may eventually lead to cirrhosis in a proportion of patients. Transplant patients on high-dose immunosuppression may have high-level intrahepatic hepatitis C viral expression, and, in this setting, the virus may induce direct cytopathic liver damage.

Data on the pathogenetic mechanisms involved in hepatic damage in acute hepatitis C are limited. Diepolder et al. (6) showed that a strong CD4+ proliferative and cytokine response to the HCV nonstructural protein 3 was correlated with viral clearance in acute HCV infection. In the chimpanzee model, Cooper et al. (5) showed that animals that progressed to chronic HCV infection had no detectable or had a narrowly focused HCV-specific CD8+ cytotoxic T lymphocyte (CTL) response, whereas animals that cleared HCV infection had early multispecific CTL responses that were durable for at least 1.5 years. These data suggest that a host immune response may play a role in determining the clinical outcome in acute hepatitis C. Whether HCV exerts any direct viral cytopathic effect in this clinical setting is not known.

CHRONIC HEPATITIS C

Chronic hepatitis C causes insidious and progressive liver damage in most patients. Histologically, features of chronic viral hepatitis, including a diffuse necroinflammatory reaction, are present. In addition, lymphoid aggregates in portal tracts, epithelial damage of small bile ducts, and microvesicular and macrovesicular steatosis of hepatocytes are also commonly seen with chronic hepatitis C. A small but increased number of hepatocytes show features of apoptotic liver cell death. Liver fibrogenesis is enhanced, which contributes to the fibrosis and cirrhosis seen in late stages of chronic hepatitis C.

Immunophenotyping of intrahepatic infiltrating inflammatory cells showed a predominance of T cells, with a significant proportion of CD4+ and CD8+ cells, suggesting that the host immune system is involved in liver disease pathogenesis (26). Expression of immune adhesion molecules was also upregulated, which may reflect active recruitment and priming of T cells in the liver of these patients (12).

Patients with mild HCV-induced liver disease have been shown to have a strong HCV-specific CD4+ proliferative response in peripheral blood compared with patients with active liver diseases (4, 13). These findings were interpreted as an indication that CD4+ response plays a role in controlling HCV infection. An alternative explanation is that HCV-specific CD4+
cells are compartmentalized in the liver of patients with active liver disease and, hence, there is a low frequency of HCV-specific CD4+ cells in peripheral blood. The latter hypothesis is supported by the demonstration that HCV-specific CD4+ clones derived from liver utilized different T cell receptors compared with those clones derived from peripheral blood (21). The infiltrating CD4+ T cells were shown to be mostly the T helper 1 (Th1) subtype, as reflected by the pattern of cytokine release by the liver-derived T cell clones generated from these patients (2). More direct evidence comes from the demonstration that Th1, but not Th2, cytokine messenger RNA was detected in the liver of patients with chronic hepatitis C (22). It is important to note that, although a Th2 response was not detected in the liver, circulating Th2 cytokine levels were found to be increased in a proportion of patients with chronic hepatitis C, suggesting that the peripheral Th2 response may be an autoregulatory mechanism that confines the Th1 response to within the liver (28). One cannot rule out that this Th2 response plays a role in viral persistence, but there is a lack of definitive evidence supporting this hypothesis.

The HCV-specific HLA class I-restricted CD8+ CTL response is also commonly detected in the liver of patients with chronic hepatitis C (23). Similar to HCV-specific CD4+ cells, the frequency of HCV-specific CD8+ CTL precursors was found to be higher in liver than in peripheral blood, suggesting active recruitment of HCV-specific CD8+ CTL in liver, the primary site of HCV infection. The detection of HCV-specific CD8+ CTL activity in liver was associated with lower levels of viremia and higher levels of disease activity, providing support that HCV-specific CD8+ CTL is involved in the control of viral infection and contributes to disease activity (24). CD8+ CTL in liver were shown to be functionally active; messenger RNA levels of both perforin-granzyme B and Fas ligand-Fas pathways were elevated in the liver of patients with chronic hepatitis C (3). The CD8+ CTL clones derived from the liver of these patients were shown to secrete tumor necrosis factor-α and interferon-γ, which may also play a role in the control of viral replication and which may contribute to liver disease (17).

A B cell response (or antibodies to HCV (anti-HCV)) has been detected in most patients with chronic HCV infection. Despite its diagnostic utility, the role of B cell response in disease pathogenesis is unknown. The observation that most patients develop chronic infection in the presence of anti-HCV suggests that such antibodies fail to induce viral clearance. In the chimpanzee model, neutralizing antibodies were shown to be highly strain specific (and even quasispecies specific) and not protective against heterologous or even autologous challenge (9, 10). From the perspective of disease pathogenesis, anti-HCV may cause liver damage by recognizing HCV antigen (or autoantigen) on the surface of infected cells or through immune complex deposition. HCV antigens have been detected in the cytoplasm of infected cells but not on cell membranes both in vivo in patients’ livers as determined by immuno-histochemistry and in vitro using a high-level recombinant vaccinia expression system (8, 19). Immunglobulin deposition was found to be uncommon in the liver of patients with chronic hepatitis C (8).

As discussed above, pathomorphological studies have shown occasional acidophilic bodies and hepatocyte dropout, features that are compatible with apoptosis in patients with chronic hepatitis C. This was confirmed by in situ terminal transferase labeling (7). As discussed above, host immune CTL-mediated pathways of apoptosis are activated, suggesting that apoptosis of liver cells may at least partly be related to the host immune defense. Whether other cytokine-mediated and cellular constitutive apoptotic pathways are involved remains to be studied. There are recent data suggesting that HCV viral proteins may modulate apoptosis.

Direct viral cytopathicity has been suggested to contribute to disease pathogenesis in yellow fever virus infection, another member of the Flaviviridae family. Histomorphological examination also occasionally revealed cells with cytopathic changes yet without adjacent inflammatory changes. The presence of a normal liver carrier with normal liver histology and the observation that isolated hepatocytes with cytopathic changes are uncommon suggest that direct viral cytopathicity does not contribute significantly to liver damage in chronic hepatitis C.

Enhanced fibrogenesis is one of the consequences of chronic hepatitis C, which may ultimately lead to bridging fibrosis and cirrhosis. The recognition that hepatic stellate cells (formerly known as lipocytes, Ito cells, or fat storage cells) play a central role in fibrogenesis suggests that this cell type may be involved in mediating the enhanced fibrogenesis seen in chronic hepatitis C (11). Needless to say, insights gained from the molecular regulation of hepatic stellate cell activation may assist in the design of better therapeutic strategies against the long-term consequences of chronic hepatitis C.

From the above discussion, one can conclude that the host immune response is activated in chronic hepatitis C. However, humoral, cellular immune, and cytokine responses do not appear to be sufficient to eradicate HCV infection in most patients. In an attempt to clear the virus from the liver, the host immune system, particularly T cell-mediated activity, contributes to hepatocellular damage. Fibrogenesis is also enhanced, and the resulting scarring and liver regeneration lead to liver cirrhosis. Direct cytopathicity does not appear to contribute significantly to hepatocellular damage in chronic hepatitis C.

The host immune response to HCV has therapeutic implications. A recent study showed that patients with high levels of HCV-specific CD8+ CTL activity had a higher chance to develop a sustained response to interferon-α therapy (25). The role of this host immune factor in determining the subsequent response to interferon-α-ribavirin combination therapy is not yet known. New therapeutic approaches that augment host cell-
lar immune responses to HCV may potentially enhance the patients’ response to interferon-α therapy.

The mechanisms that lead to the formation of lymphoid aggregates in portal tracts, epithelial damage of small bile ducts, and microvesicular and macrovesicular steatosis of hepatocytes are not clear. The quasispecies nature of HCV may provide a constant antigenic challenge, resulting in the formation of lymphoid follicles locally in the liver. HCV has not been detected in bile duct epithelial cells in most in situ localization studies. Whether the small bile duct damage is secondary to immune damage is not known. A recent in vitro study reported steatosis in cells expressing HCV core antigen (1). Other investigators expressing HCV core antigen derived from different isolates did not observe steatosis in cell-based expression or transgenic mice. Whether hepatocellular steatosis is associated with infection with certain HCV strains remains to be studied.

**HCV RECURRENCE IN TRANSPLANT RECIPIENTS**

In a small subset of transplant recipients who had HCV infection or recurrence, HCV induces a unique pattern of liver failure that was clinically characterized by marked cholestasis and coagulopathy and histologically characterized by hepatocellular ballooning and with minimal inflammatory reaction (20, 29). These patients had very high levels of HCV viremia and high levels of hepatic expression of HCV RNA. This pattern of clinically rapid graft failure and the histological disease pattern resembled fibrosing cholestatic hepatitis seen in liver transplant recipients with severe hepatitis B recurrence in which high-level expression of hepatitis B viral antigens was believed to cause direct cytopathic changes in the infected liver (18). Cell lines expressing HCV structural proteins have been shown to exhibit endoplasmic reticulum dilation and proliferation as well as cell ballooning (30). However, transgenic mice expressing high levels of HCV structural proteins were found to have normal liver histology (15). It is tempting to speculate that the direct cytopathic effect of HCV is only evident in cells expressing very high levels of HCV. It is also possible that the expression of high levels of HCV may enhance cellular susceptibility to cytokines or other mediators of liver cell injury. If high-level expression of HCV is the key factor in liver damage in this clinical setting, potent antivirals against HCV, when available, should be chosen for therapeutic intervention.

**EXTRAHEPATIC MANIFESTATIONS**

A number of extrahepatic manifestations, including mixed cryoglobulinemia, lymphoma, development of anti-liver-kidney microsomes (anti-LKM), membrane-proliferative glomerulonephritis, Sjogren’s syndrome, and so forth, have been suggested to have an association with chronic HCV infection. The pathogenetic mechanisms involved in these manifestations are not clear. The observation that autoantibodies, including anti-LKM, anti-thyroid, and anti-GOR, are commonly detected in patients with chronic HCV infection suggests that a B cell response to HCV may contribute to extrahepatic and autoimmune immunopathology. Interestingly, more than half of patients with chronic HCV infection showed marked expansion of CD5+ B lymphocytes in their peripheral blood (27). Activation of this B cell subset has been linked to autoimmune diseases, including rheumatoid arthritis (14). Selective activation of this B cell subset may be linked to the development of autoimmunity and possibly B cell lymphoma in patients with chronic hepatitis C. This is an important area that has not been explored, and further studies are needed to define the mechanisms involved.

In conclusion, current data suggest that, in chronic hepatitis C, a host cellular immune response is involved in the control of viral replication and contributes to hepatocellular damage. Because the host immune response is not able to clear the viral infection, continuous liver damage and regeneration, together with enhanced fibrogenesis, lead to cirrhosis in a proportion of patients with late-stage chronic hepatitis C. HCV does not appear to induce clinically significant direct cytopathic damage in chronic hepatitis C. However, this mechanism may be responsible for liver damage in patients on high-dose immunosuppression and with high-level intrahepatic HCV expression.

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Present address and address for reprint requests: J. Y.-N. Lau, Dept. of Antiviral Therapy, K-15-4-4650, Schering-Plough Research Institute, 15 Galloping Hill Road, Kenilworth, NJ 07033.

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