

Replication of Hepadnaviruses in Transient and Chronic Infections

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Hepatitis B virus (HBV) and related hepadnaviruses infect primarily hepatocytes, a self-renewing cell population. Infection is followed by conversion of the relaxed circular viral DNA genome (rcDNA) into covalently closed circular DNA (cccDNA). cccDNA is localized to the nucleus, where it is transcribed to form the viral mRNA. One of the two largest mRNA molecules, the pregenome, is packaged into viral nucleocapsids, located in the cytoplasm, and reverse transcribed to produce progeny rcDNA. Some of the newly made rcDNA is transported to the nucleus to amplify the cccDNA copy number to 10-50 per hepatocyte. Further increases in cccDNA copy number are blocked, probably because viral envelope proteins direct all additional, newly made rcDNA into the secretory pathway to produce progeny virus. Infected hepatocytes produce large numbers of infectious virions resulting in the rapid spread of infection throughout the entire liver (Meier et al., 2003; Summers et al., 2003). Hepadnavirus infection can be either transient (acute) or chronic infections. Since infection *per se* is non-cytopathic, the liver damage that occurs during both acute and chronic infection is immune-mediated. Interestingly, resolution of transient infections often fails to initiate until after 100% of hepatocytes are already infected (Summers et al., 2003).

How an infection can be cleared by the immune system following infection of all hepatocytes is uncertain. The answer to this question may provide new strategies for treatment of chronic infections. It is currently thought that immune-mediated cell death plays a critical role by removing hepatocytes containing cccDNA and stimulating the division of other infected hepatocytes to maintain liver mass (Summers et al., 2003). Cell division is thought to destabilize cccDNA and/or to facilitate its loss via dilution. Antiviral cytokines, including interferon-alpha (IFN- α), interferon-gamma (IFN- γ), and tumor necrosis factor-alpha (TNF- α), are thought to act to destabilize nucleocapsids containing RI DNA and thereby to prevent the formation of new cccDNA. Destabilization of cccDNA by cytokines has not yet been demonstrated but has been speculated to occur (Murray et al., 2005). It is unclear if anti-viral antibodies, in particular anti-HBV surface antibodies that can bind to and neutralize circulating virions and prevent reinfection of newly divided hepatocytes, play important roles during clearance or are only needed later to prevent re-emergence of the virus.

Despite the obvious clearance of virus during resolution of transient infection, viral DNA often persists for months to years at a low level, resulting in residual HBV infection. Residual HBV infection is accompanied by persistent cytotoxic T lymphocyte (CTL) responses, with a slightly increased risk of liver cancer; moreover, infection can reactivate following immunosuppression or liver transplantation. Recent data suggest that cccDNA is the key form of viral DNA present during residual duck hepatitis B virus (DHBV) infection (Le Mire et al., 2005), highlighting the stability of the cccDNA molecule.

HBV causes chronic infection in 5-10% of adults and 30-90% of children infected when they are less than 5 years of age. Chronic HBV infection develops because the immune response fails to clear or completely control virus replication. However, it is clear that targeting of infected hepatocytes still occurs, as chronic infection is associated with persistent immune attack on infected hepatocytes that

results in mild to severe liver disease. Chronic infection is also linked to increased rates of fibrosis, cirrhosis, and primary liver cancer, hepatocellular carcinoma (HCC).

HCCs are thought to arise rarely from the thousands of foci of altered hepatocytes that appear in the liver of chronically infected patients over time. Thus, the foci are in some sense preneoplastic. The reason why these foci emerge is unclear. One possibility is suggested by reports that the foci of altered hepatocytes express either no or only low levels of virus antigens. Hepatocytes that stopped expressing the virus would have a selective growth advantage because, even if their growth rate were the same as surrounding hepatocytes, they would die at a lower rate because they would not be effectively targeted by antiviral CTLs. Consistent with this theory, the liver of woodchucks with chronic woodchuck hepatitis virus (WHV) infection was shown to contain at least 100,000 foci of greater than 1,000 cells that arose subsequent to infection with WHV (Mason et al., 2005). Thus, a major role of the immune response in emergence of HCC may be the persistent selection for emergence of hepatocyte clones that, for any reason, have lost the ability to replicate the virus. If so, the most important factor in emergence of HCC may be the cumulative hepatocyte turnover that occurs during the course of a chronic infection.

In summary, there are several major unresolved issues in HBV pathogenesis:

1. How are HBV infections cleared from the liver, and what are the relative roles of hepatocyte death vs. cytokine-mediated effects during the resolution of transient infection?
2. What treatment strategy for chronic infections will achieve elimination of HBV from the vast majority of hepatocytes and control of the residual virus that remains behind? Are there non-cytopathic pathways for elimination of cccDNA? What other treatment strategies might work?
3. How does chronic infection lead to hepatocellular carcinoma?

References

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