Animal models for HCV and HBV studies

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The narrow host range of infection and lack of suitable tissue culture systems for the propagation of hepatitis B and C viruses are limitations that have prevented a more thorough understanding of persistent infection and the pathogenesis of chronic liver disease.

Despite decades of intensive research and significant progresses in understanding of viral hepatitis, many basic questions and clinical problems still await to be resolved. For example, the HBV cellular receptor and related mechanisms of viral entry have not yet been identified. Little is also known about the function of certain non-structural viral products, such as the hepatitis B e antigen and the X protein, or about the role of excess hepadnavirus subviral particles circulating in the blood stream during infection. Furthermore, the molecular mechanisms involved in the development of hepatocellular carcinoma and the role of the immune system in determining the fate of infection are not fully understood. The reason for these drawbacks is essentially due to the lack of reliable cell-based in vitro infection systems and, most importantly, convenient animal models.

This lack of knowledge has been partially overcome for hepatitis B virus (HBV), by the discovery and characterization of HBV-like viruses in wild animals while for hepatitis C virus (HCV), related flaviviruses have been used as surrogate systems. Other laboratories have developed transgenic mice that express virus gene products and/or support virus replication. Some HBV transgenic mouse models develop fulminant hepatitis, acute hepatitis, or chronic liver disease after adoptive transfer, and others spontaneously develop hepatocellular carcinoma (HCC). Among HCV transgenic mice, most develop no disease, but acute hepatitis has been observed in one model, and HCC in another. Although mice are not susceptible to HBV and HCV, their ability to replicate these viruses and to develop liver diseases characteristic of human infections provides opportunities to study pathogenesis and develop novel therapeutics

In the search for the mechanism of hepatocarcinogenesis in hepatitis viral infection, two viral proteins, the core protein of hepatitis C virus (HCV) and the HBx protein of hepatitis B virus (HBV), have been shown to possess oncogenic potential through transgenic mouse studies, indicating the direct involvement of the hepatitis viruses in hepatocarcinogenesis. This may explain the very high frequency of HCC in patients with HCV or HBV infection.

Chimpanzees remain the only recognized animal model for the study of hepatitis C virus (HCV). Studies performed in chimpanzees played a critical role in the discovery of HCV and are continuing to play an essential role in defining the natural history of this important human pathogen. In the absence of a reproducible cell culture system, the infectivity titer of HCV challenge pools can be determined only in chimpanzees. Recent studies in chimpanzees have provided new insight into the nature of host immune responses-particularly the intrahepatic responses-following primary and secondary experimental HCV infections. The immunogenicity and efficacy of vaccine candidates against HCV can be tested only in chimpanzees. Finally, it would not have been possible to demonstrate the infectivity of infectious clones of HCV without chimpanzees. Chimpanzees became infected when RNA transcripts from molecular clones were inoculated directly into the liver. The infection generated by such transfection did not differ significantly from that observed in animals infected intravenously with wild-type HCV. It furthermore permits true homologous challenge in studies of protective immunity and in testing the efficacy of vaccine candidates. Finally, this in vivo transfection system has made it possible to test for the first time the importance of genetic elements for HCV infectivity.

Although chimpanzees are the only animals fully permissive for HBV infection, their use for research purpose is severely limited by the high costs and strong ethical constrains. The only alternative source of HBV-permissive hepatocytes is the Asian tree shrew Tupaia belangeri. Though experimental infection of these squirrel-like mammals, phylogenetically related to primates, results only in a mild, transient replication, primary hepatocytes isolated from T. belangeri turned out to be a reliable tool for in vitro HBV infection experiments.

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Along with invaluable infection studies in chimpanzees, avian and mammalian HBV-related viruses continue to offer ample opportunities for studies in naturally occurring hosts. In general, most of our progresses in hepatitis B virus research are based on infection studies with two HBV-related animal viruses: the woodchuck HBV (WHV), which infects the Eastern American woodchuck (Marmota monax), and the duck HBV (DHBV), which infects Peking ducks. Both animal models have been essential for understanding various steps of viral life-cycle and factors involved in establishment of virus infection, persistence and hepatocarcinogenesis.

Studies performed over the last ten years with HBV-replicating transgenic mice demonstrated that this small animal model is suitable to evaluate the impact of antiviral treatment strategies on HBV replication and for immunological studies upon induction of cytokines or adoptive transfer of HBV-specific cytotoxic T lymphocytes (CTLs). More recently, mouse models, based on transfection of recombinant adenoviral vector or hydrodynamic injection of naked DNA, have been developed to investigate mechanisms of viral clearance. Compared with transgenic mice, in vivo transfection systems should enable fast comparison of viral mutants for their replication competence. Nevertheless, for various reasons none of the above mentioned models are ideal, since all natural hosts of HBV-related viruses are of out-bred origin and their immune systems have not been characterized.

Genetic alterations and epigenetic changes in hepatocarcinogenesis

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Hepatocarcinogenesis as hepatocellular carcinoma (HCC) is associated with background of chronic liver disease usually in association with cirrhosis, marked hepatic fibrosis, hepatitis B virus (HBV) and/or hepatitis virus (HCV) infection, chronic inflammation, Aflatoxin B1(AFB1) exposure, chronic alcoholism, metabolic disorder of the liver and necroinflamatory liver disease. Hepatocarcinogenesis involve two mechanisms, genetic alterations (with changes in the cell's DNA sequence) and epigenetic changes (without changes in the cell's DNA sequence), but changes in the pattern of gene expression that can persist through one or more generations (somatic sense). Hepatocarcinogenesis is associated with activation of oncogenes and decreased expression of tumor suppressor genes (TSG); include those involved in cell cycle control, apoptosis, DNA repair, immortalization and angiogenesis. AFB1 is metabolized in the liver into a potent carcinogen, aflatoxin 8, 9-epoxide, which is detoxified by epoxide hydrolase (EPHX) and glutathione S-transferase M1 (GSTM1). A failure of detoxification processes can allow to mutagenic metabolite to bind to DNA and inducing P53 mutation. Genetic polymorphism of EPHX and GSTM1 can make individuals more susceptible to AFB1. Epigenetic inactivation of GSTP1 by promoter hypermethylation plays a role in the development of HCC because, it leads that electrophilic metabolite increase DNA damage and mutations. HBV DNA integration into the host chromosomal DNA of hepatocytes has been detected in HBV-related HCC. DNA tumor viruses cause cancer mainly by interfering with cell cycle controls, and activating the cell's replication machinery by blocking the action of key TSG. HBx protein is a potent co-transactivator of viral and cellular promoters such as c-yuck and c-fos. Binding HBx protein to the p53 protein may interrupt p53 induced apoptosis and may inhibit DNA repair during hepatocarcinogenesis. Liver infection may lead to enhanced cell proliferation, in presence of DNA damage from AFB1, result in increased mutations. Genetic alterations and rearrangements are present in the early steps in hepatocarcinogenesis. Genetic alterations, including two different mechanisms relate to chromosomal instability (CIN) and CpG island methylation. Genetic alterations and epigenetic changes in oncogenes and tumor suppressor genes may cause gain of functions or loss of functions respectively. HCC accu-

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