Hepatitis B Virus Propagated in a Rat Hepatoma Cell Line Is Infectious in a Primate Model

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The human hepatitis B virus (HBV) produced by a rat hepatoma cell line through transfection with HBV DNA is infectious in the human primate model—chimpanzee. Since hepadnaviruses are known to have an extremely narrow host range, our results support the idea that the host species barrier of HBV infection resides on the penetration/adsorption step rather than any postpenetration intracellular event during the virus life cycle.

A rat hepatoma cell line (Q7) of Morris hepatoma origin was transfected with a plasmid containing the tandem dimer genome of human hepatitis B virus (HBV). Several single-cell clones were found to accumulate high levels of secreted hepatitis B surface antigen (HBsAg) and e antigen (HBeAg) in the medium. DNA polymerase activity and 42-nm Dane-like particles along with an excess number of 22-nm subviral particles were observed in the medium of one of the clones designated as Q7 HBV-21. Southern blot analysis exhibited both intracellular and extracellular HBV replicative intermediates including relaxed circular and single-stranded forms. This indicated that HBV was in active replication and complete virion was produced. These results demonstrated the permissiveness of rat hepatocyte to the replication of human virus. Thus, the species barrier of HBV host range probably resides at the penetration/adsorption step during the early phase of the viral life cycle.

To assess the infectivity of the Dane-like particles produced by the rat hepatoma cell line in culture, a susceptible chimpanzee was inoculated with the medium collected from Q7 HBV-21. Ten milliliters of cell-free supernatant from a 5-day-old culture medium of Q7 HBV-21 cell line was intravenously injected into a naive chimpanzee. Markers for hepatitis and HBV serology and its DNA replicative intermediates were monitored throughout the course of the experimental infection. Serum aminotransferase (ALT) level, a liver-specific enzyme, was monitored weekly. HBV-specific markers including HBsAg and HBeAg, IgM and IgG antibodies to hepatitis B virus core antigen, hepatitis B e antigen (HBeAg), and antibody (HBeAb) were also monitored. Southern blot analysis of DNA samples from pelleted viral particles from serum was probed with vector-free, gel-purified 3.1-kb HBV DNA. The subtype specificity of HBV produced by Q7 HBV-21 and by the infected chimpanzee was determined by restriction nuclease mapping of the polymerase chain reaction (PCR) products as described by Cheung et al. (6).

Infectivity of human hepatitis B virus produced from the HBV DNA transfected rat hepatoma cell line (Q7 HBV-21) was demonstrated in the chimpanzee primate model (Fig. 1). After intravenous injection of 10 ml of cell-free Q7HBV-21 culture supernatant into the susceptible chimpanzee, characteristic hepatitis developed with the appearance of HBsAg on the 3rd week and elevation of serum alanine aminotransferase (ALT), beginning in the 9th week and peaking on Week 15 before returning to normal after the 18th week. HBsAg remained detectable for 10 weeks followed by a window period of 3 weeks. Anti-HBs antibody was detectable from the 18th week. Antibody to hepatitis B core antigen was detected on the 7th week, the total antibody to HBcAg was elevated throughout the course of the study while the IgM antibody appeared only between Week 7 and Week 16. Transient HBeAg positivity was seen between Week 5 and Week 11 followed by seroconversion to anti-HBe on Week 14. This pattern of serological events is typical for acute human hepatitis B infection in the chimpanzee model (7). Using a vector-free 3.1-kb HBV DNA probe, Southern blot analysis demonstrated that HBV-specific DNA replicative intermediates in relaxed circle (RC) and single-strand (SS) forms could be detected in the serum from Week 5 to Week 11 with a peak on Week 8 (Fig. 2; the data from Weeks 10 to 14 were not shown). The appearance of HBV DNA in the sera coincided with the

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presence of HBeAg. There was no demonstrable genetic alteration of HBV as detected by strain analysis using the PCR procedure (data not shown). HBV both in the inoculum and in the chimpanzee serum were both ayw subtype strain as was the original transfecting DNA (1).

Previously, the infectivity of HBV and duck hepatitis B virus produced in tissue culture using human hepatoma or hepatoblastoma cell lines has been demonstrated (8-11). However, HBV produced in tissue culture using a non-human hepatocyte has not been previously shown. Our results indicate that infectious human HBV was produced by transformed rat hepatoma cells. The short incubation period suggests that the titer of infectious HBV was rather high (12). Several attempts have been made to propagate human hepatitis B virus in a transgenic mouse animal model (13, 14). Although HBV DNA replication can be detected in this system, no matured virion particles can be detected in the liver or serum of the transgenic animals. Hepadnaviruses are known to have a very narrow host range (2-4). Our results appear to be consistent with the idea that the species barrier of HBV infection resides on the step of adsorption/penetration of the target hepatocytes. It argues against the notion that the species barrier occurs in the postpenetration intracellular events.

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