Hepatitis B virus genotype C infection promotes polyploidization and growth arrest in human hepatocytes of humanized chimeric mouse liver

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BACKGROUND & AIMS: Several studies performed in established cell lines and transgenic mice have reported that expression of hepatitis B virus(HBV) proteins, such as surface and X proteins, affect hepatocyte proliferation and induce development of hepatocellular carcinoma.
However, the manner in which HBV infection affects phenotypes of normal human hepatocytes (HHs), which are natural hosts for HBV, has not been elucidated. In the present study, we examined the effects of HBV infection on the morphology and proliferation of HHs using chimeric mice with humanized livers. METHODS: Commercially available cryopreserved HHs were transplanted into urokinase-type plasminogen activator cDNA-transgenic/severely combined immunodeficient (cDNA-uPA/SCID) mice that were 3 weeks old. Seven-week-old chimeric mice were inoculated with HBV genotype (Gt.) A and C (10^6 copies/animal). At 12 and 20 weeks after infection, HHs were collected by collagenase perfusion and examined by microscopic observation and flow cytometric analyses, to evaluate the effect of HBV-infection on the cell size, ploidy, and ratio of binuclear HH. Pegylated interferon (PEG-IFN) alpha-2a was administered (30 μg/kg, twice/week) to chimeric mice infected with HBV Gt. C for 8 weeks (19-27 weeks old), and HHs were isolated from the mice, to confirm the effect of the antiviral treatment on the morphological changes induced by HBV infection. To examine the effects of HBV infection on HH proliferation, HHs were isolated from naïve and HBV Gt. A, and C-infected chimeric mice and transplanted into 3-week-old cDNA-uPA/SCID mice. RESULTS: Compared with naïve HHs, HBV Gt. C infection induced robust HH hypertrophy, and increased the number of binuclear HHs at 12 weeks after infection. However, Gt. A infection induced only a slight HH hypertrophy. At 20 weeks after infection with HBV Gt. C, the HHs were further enlarged, and many atypical nuclei were observed. Flow cytometric analysis and microscopic observation revealed that ploidy increased with the duration of infection. PEG-IFN alpha-2a treatment for 8 weeks reduced cell size and inhibited the ploidy increase caused by HBV infection. At 3 weeks after transplantation, the chimeric mice injected with Gt. C-infected HHs showed significantly lower blood human albumin levels than the mice injected with naïve or Gt. A-infected HHs. It indicates that the HH proliferation was specifically inhibited by Gt. C infection. CONCLUSIONS: These results suggest that HBV Gt. C infection not only induced hepatocyte hypertrophy and ploidy increase, but also inhibited hepatocyte proliferation in vivo.