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The relationship between hepatitis b virus and apoptosis in humans and in a transgenic mouse model

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ABSTRACT

Hepatitis B virus (HBV) has been found to be highly endemic in Africa and south east Asia. In southern Africa, subgenotype A1 and genotype D prevail while in south east Asia genotype B and C predominate. Infection with HBV can lead to a wide spectrum of clinical presentations ranging from an asymptomatic carrier state to self-limited acute or fulminant hepatitis to chronic hepatitis with progression to cirrhosis and hepatocellular carcinoma (HCC). It has been shown that viral factors as well as a number of host and environmental factors can influence the course of HBV infection. Development and progression of various liver diseases are associated with either an increase or decrease in hepatocyte apoptosis. Dysregulated apoptosis itself may be a fundamental feature of most acute and chronic human liver diseases.

The purpose of this study was to characterise the subgenotype A1 and genotype D HBV infection, prevailing in South Africa. To control for the influence of host factors on HBV infection as well as to avoid the use of *in vitro* cell lines, such as Huh-7, that have defective apoptotic pathways, the *in vivo* urokinase plasminogen activator severe combined immunodeficient (uPA-SCID) transgenic mouse model was utilised. The HBV infection of the transgenic mice infected with HBV positive sera containing either subgenotype A1 wild-type, subgenotype A1 with the G1862T mutation, subgenotype A2 or genotype D, was compared.

For the first time, we were able to demonstrate the successful infection of the uPA-SCID transgenic mouse model with subgenotype A1 of HBV. The successful establishment of the *in vivo* HBV infection with different genotypes or subgenotypes in the uPA-SCID transgenic mice was demonstrated by the increase of HBV DNA levels, the presence of cccDNA and HBV transcripts as well as the detection of the core and/or surface HBV antigens in the liver tissue of the chimeric mice. Differences between the HBV infections with the various genotype/subgenotypes were observed. Subgenotype A1 with the G1862T mutation showed the earliest detection and therefore highest levels of cccDNA as well as the highest HBV DNA levels when compared to the other strains. The highest HBV DNA levels were recorded for the subgenotype A1 G1862T infected transgenic mouse followed by genotype D, subgenotype A2 and the lowest levels observed in the subgenotype A1 wild-type infected

transgenic mouse. HBsAg was also only detected in the livers of mice infected with subgenotype A1 with the G1862T mutation. HBcAg staining in the chimeric liver was positive when the mice were infected with genotype D, which concurs with previous observations that genotype D is characterised by high HBcAg expression. Subgenotype A1 with the 1862 mutant showed the highest levels of apoptosis as a result of the abnormal precore precursor protein accumulation shown to be associated with this 1862 missense mutation. Thus different genotypes and subgenotypes as well as variations within genotypes can influence HBV infection. Moreover, the results of these experiments in the immunocompromised chimeric mice, grafted with liver cells from a single donor, suggests that even when host and environmental risk factors are controlled for, the subgenotype or genotype can influence the course of infection.

The limitations of the uPA-SCID transgenic mouse model include the lack of an immune system and the short life-span of the animal; therefore a population based study was carried out to investigate the influence of host factors on HBV infection in various disease groups. The study cohort comprised 635 serum samples from South Africa, China and Japan. Of these samples, 564 were HBsAg-positive and the remaining 71 HBsAg-negative, HBV DNA negative controls. The study cohort included asymptomatic carriers; chronically infected HBV patients as well as patients with HBV associated HCC. Possible associations were determined between HBV genotype, HBV viral load, apoptosis levels, disease group and the age and gender of the patient where available. Apoptosis levels were quantified by the measurement of cleaved cytokeratin 18 (M30) in serum.

Patients infected with genotype C or subgenotype A1 were shown to possess a higher odds ratios of developing HCC compared to subgenotype B2 or genotype D, respectively. Significantly higher HBV viral loads were observed in genotype C compared to subgenotype B2. Among the Asian cohort, it was also shown that the male gender was positively associated with high viral loads in HCC patients. Moreover, a positive association between higher HBV viral load levels and HCC in the South African cohort was observed. Male gender, older age, HBV viral load, subgenotype A1 and the presence of the G1862T mutation were shown to be positively and significantly associated with higher levels of apoptosis. In this study it was discovered that the levels of cleaved cytokeratin 18 could potentially be used as a biomarker for the severity of HBV infection because a significant difference was

observed with the apoptosis levels between the asymptomatic and HCC patient disease groups.

We conclude that even when the influence of host and environmental factors is controlled for, as is the case in the chimeric mouse model, the HBV genotype can affect the progression of infection. Moreover, it was shown in the population based study that the effect of HBV genotype on the outcome of HBV infection can be influenced by host factors. The subgenotype A1 G1862T mutation was shown in both studies to affect both HBV infection and apoptosis. This suggests that HBV variants should be investigated to ascertain their potential impact on the course of HBV infection as it may differ from the wild-type. Apoptosis was shown to be associated with HBV infection in both studies and could possibly be an ideal marker of the progression of HBV infection.

These findings are important in helping us to understand factors influencing the course of HBV infection. We have therefore shown in both the studies that differences do exist between the South African subgenotype A1 and genotype D, and that these differences should be taken into consideration for the future evaluation of HBV infection and treatment of South African HBV infected patients. Moreover, cleaved cytokeratin 18 may provide an ideal surrogate marker for HBV disease progression and monitoring.