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Identification of Hepatitis B Virus (HBV) PreSurface and Surface Mutations among HIV Positive Individuals with Occult HBV Infection

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Introduction

Hepatitis B virus (HBV) is an important cause of morbidity and mortality among HIV-positive individuals. Before antiretroviral therapy (ART), HIV patients were more likely to develop chronic HBV infection due to impaired immune function. Now ART is more effective, patients are living longer and liver disease is becoming more apparent. Multiple distinct HBV genotypes have been described. In addition, multiple viral variants exist within an individual (viral quasispecies). Occult HBV is defined as persistent low level HBV replication in the absence of detectable HBsAg. Antibodies against core are usually the only detectable serologic marker, though serologically negative cases exist. Prevalence of occult HBV infection in HIV+ cohorts ranges from 0-54%. Virologic characterization of occult HBV, particularly during HIV co-infection, has not been well studied. We hypothesized that unique mutations are present in HBV DNA from subjects with occult HBV infection.

Methods

Serum samples were tested for HBV DNA by real-time PCR and serologic markers of infection by ELISA. HBV DNA levels ranged from 113 to 2.6×10^6 IU/mL for chronic HBV infections and from 109 to 0.76×10^6 IU/mL for occult HBV infections. 31 chronic HBV infections and 12 occult HBV infections were identified. To identify mutations unique to occult infection, the PreSurface (PreS) and Surface (S) regions from 30 chronic and 6 occult HBV infections were amplified (Table 1: patient demographics). Region-specific nested PCR assays were developed for HBV PreS (542 bp) and S (356 bp) (Figure 1, pink/red) that amplify multiple HBV genotypes at varying DNA levels. Phylogenetic analysis was performed to determine the HBV genotype and quasispecies parameters such as genetic distance (GD), entropy, and dN/dS ratio were calculated. Sequence analysis comparing occult sequence to genotype matched GenBank sequences and chronic sequences was performed to identify occult-associated mutations.

	ALT	Age	Race	Gender
Chronic	55.7 (17 – 140)	36.3 (21.2 – 58)	46.7% African-American 43.3% Caucasian 6.7% Other 3.3% Hispanic	93.3 % male
Occult	36.5 (23 – 87)	35.0 (26.7 – 42.2)	50% African-American 33.3% Caucasian 16.7% Other	83.3 % male

Table 1

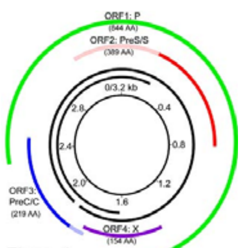

Figure 1

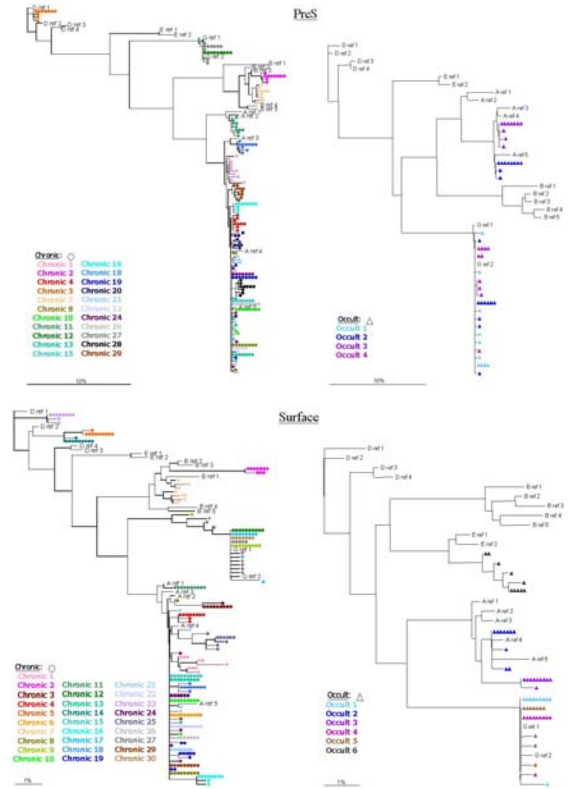

Figure 2

Results

Phylogenetic trees for the PreS (top) and S (bottom) regions of HBV (Figure 2) identified genotypes A, B, D, E and G. Occult 2, was identified as a dual infection in the PreS region consisting of both genotypes A and G (blue triangles). On average, entropy calculations were higher for PreS (PS) than S (Table 2). The average dN/dS ratio was above 1 for the occult S region, indicating positive selection pressure acting on this region (blue box). Sequence analysis identified novel mutations in subjects with occult HBV infection. In the PreS region (Figure 3A), 6 new mutations were identified for genotype A and 13 for genotype G. In the S region (Figure 3B), 3 new mutations were identified for for genotype A, 2 for genotype E and 6 for genotype G, as well as several previously described in the literature.

	Avg GD	Avg Entropy	Avg dN/dS		Avg GD	Avg Entropy	Avg dN/dS
Chronic PS	0.0023	0.5086	0.4374	Chronic S	0.0013	0.2711	0.6495
Occult PS	0.0023	0.5354	0.8282	Occult S	0.0017	0.2951	1.2116

Table 2

A

PreS1		Newly Identified				
	Genotype A		G59R	N108Y	S109P	
	Genotype G	M1I	S16P	F23S	D26Y	D41N
		P88L	P93L	R102G	T105I	P106S
PreS2		Newly Identified				
	Genotype A		S28C	H41Q	D51G	
	Genotype G		L19P	V34A	T36A	

B

	Previously Described			Newly Identified					
Genotype A	M103I	K122R	G145A	Y72H	I82T	A128T			
Genotype E	F85C	Y100S	G145R	Y100F	L127P				
Genotype G				S55P	P62L	F80S	I86V	L95W	S136P

C

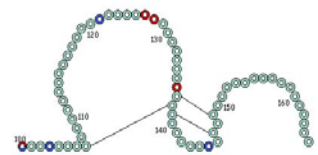

Figure 3

Mutations highlighted in Figure 3B (blue - previously described; red - newly identified) are shown in Figure 3C in the predicted secondary structure of the antigenic determinant loop of HBsAg. Lines represent predicted disulfide bonds and circles represent amino acid positions 100-165 of HBsAg.

Conclusion

Several mutations were identified in the PreS and surface regions from subjects with occult HBV infection. We speculate that these amino acid changes may alter the secondary structure of HBsAg, inhibiting antibody binding and / or contributing to the lack of detectable HBsAg during occult HBV infection. In the future, we will continue to characterize occult HBV infection by: 1) Identifying mutations throughout the HBV genome associated with occult infection; 2) Examining 3 - D HBsAg structure; and 3) Evaluating changes in HBsAg production and cellular localization.

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