HBV surface antigen glycan isomer (HBsAgGi), correlates with HBV-DNA and HBV-RNA virions

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ABSTRACT:

Background: In HBV patients' sera, infectious particles containing HBV-DNA (Dane particles) or HBV-RNA particles are enveloped with hepatitis B virus surface antigen (HBsAg). Infectious particles contain L-, M-, and S-HBsAg, while non-infectious sub-viral particles (SVPs) mainly contain S-HBsAg. Because SVPs present much more than infectious particles, S-HBsAg is the most abundant in all HBV particles. Thus, general HBsAg test using S-HBsAg antibodies cannot distinguish infectious and non-infectious particles. Previously, we found that *O*-glycosylated M-HBsAg was enriched in HBV-DNA particles. In this study, we developed and examined a new antibody to detect infectious particles, which should contain *O*-glycosylated M-HBsAg, called as HBsAg glycan isomer (HBsAgGi).
Methods and Results: Western analysis indicated that HBsAgGi antibody recognized M-HBs modified with *O*-glycan but not L-HBs without *O*-glycan on the PreS2 of genotype C. Immunoprecipitation (IP) experiments confirmed that both HBV-DNA and HBV-RNA-containing particles were immunoprecipitated by HBsAgGi antibody. HBsAgGi localized in ER to Golgi in MHBs-expressing cells. In treatment naïve chronic hepatitis B patients, serum HBsAgGi level was significantly correlated with the HBV-DNA level (p=0.002, n=32). However, HBsAgGi level was not associated with HBV-DNA level after NA treatment, which is consistent with the IP results.
Conclusions: *O*-glycosylated M-HBsAg is unique to genotype C infectious virions. New HBsAgGi antibody recognizes infectious HBV virions containing HBV-DNA or HBV-RNA, indicating that the infectious particles with HBsAgGi are generated through glycosylation pathway. Furthermore, NA treatment altered HBV-DNA and HBV-RNA status but not much HBsAgGi, suggesting that HBsAgGi is an useful monitor of infectious HBV particles during therapy.

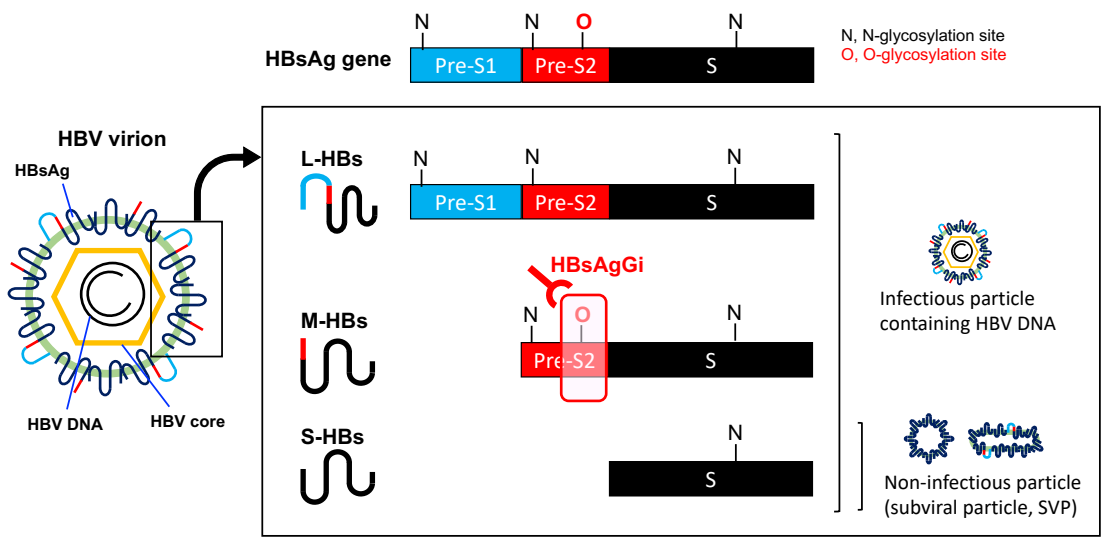
INTRODUCTION

Upon HBV infection, non-infectious particles are dominantly present than infectious particles containing HBV DNA (Dane particles) in patients. In contrast to non-infectious particles mainly containing S-HBsAg, Dane particles contain all L-, M-, and S-HBsAgs. General HBsAg test using antibodies for S-HBsAg detect all HBV particles. To distinguish infectious particles and non-infectious subviral particles, we generated M-HBsAg specific antibody. Because a lectin recognizing *O*-glycans could enrich DNA-containing particle, *O*-glycosylated M-HBsAg determined by mass spectrometry was targeted. This study aimed to examine a new antibody recognizing *O*-glycosylated M-HBsAg [HBsAg glycan isomer (HBsAgGi)] if it can detect infectious HBV particles containing HBV-DNA and/or HBV-RNA.

RESULTS

- PreS2 peptides with *O*-glycans generated a genotype C specific antibody.
- HBsAgGi antibody recognized M-HBs but not L-HBs, which is not modified with *O*-glycan on the PreS2 of genotype C.
- Mutations in *O*-glycosylation site or removal of *O*-glycans resulted in decrease of the recognition by HBsAgGi antibody.
- HBsAgGi localized in ER to Golgi in M-HBs expressing cells.
- In treatment naïve CHB patients, serum HBsAgGi level was higher in HBe-positive patients (EPH) than HBe-negative patients (ENH) at baseline.
- HBsAgGi levels were significantly correlated with the HBV-DNA level (n=32, *p*=0.002).
- HBsAgGi antibody can immunoprecipitate HBV-RNA-containing particles. Percentile of HBV-RNA particles would increase after NA therapy.

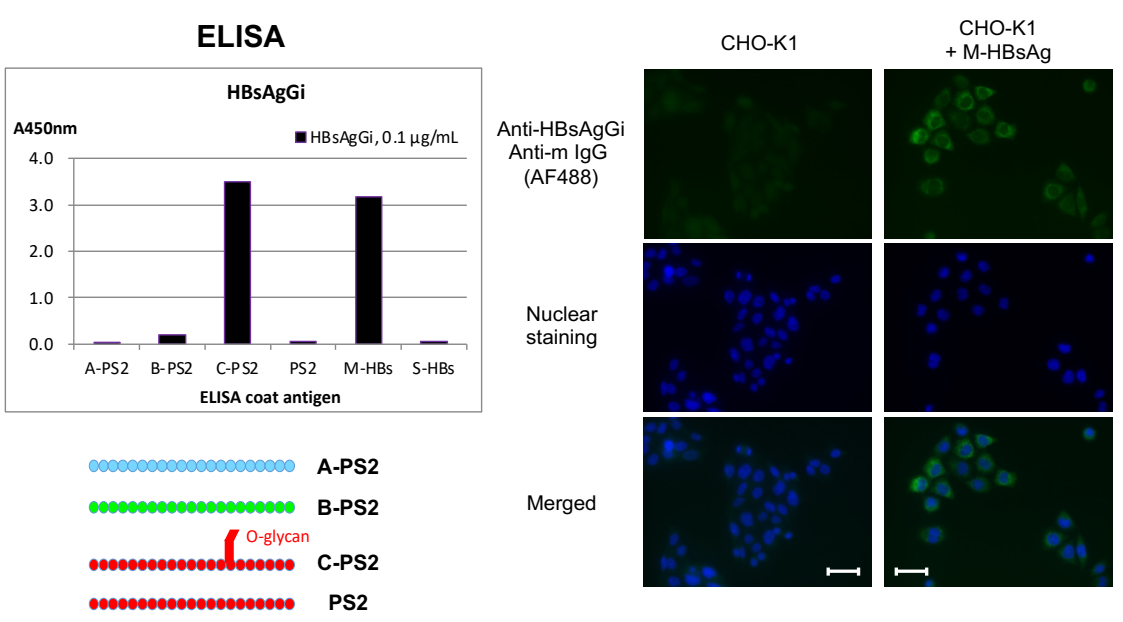
HBsAgGi antibody recognizes M-HBsAg modified with *O*-glycans in genotype C HBV virion



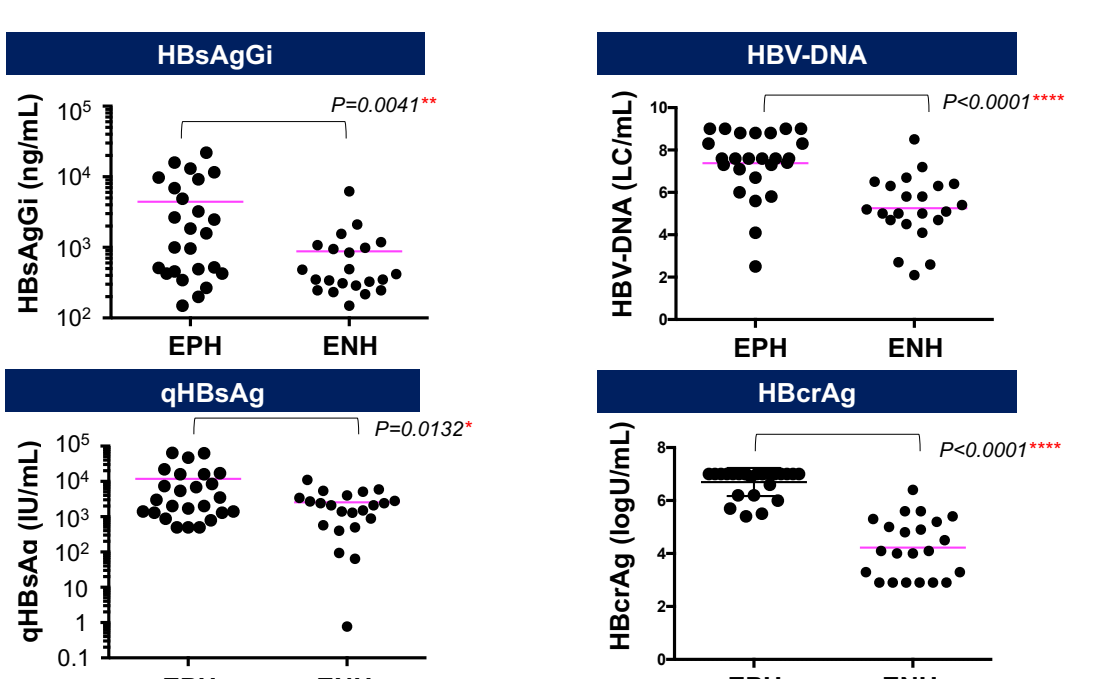
METHODS AND MATERIALS

To generate HBsAgGi antibodies, PreS2 glycopeptides modified with *O*-glycans were syntehsized. To characterize the HBsAgGi antibody, western blotting, immunostaining, and ELISA were performed. To investigate clinical utility of the HBsAgGi, sera of chronic hepatitis B (CHB) patients before and after nucleos(t)ide analog (NA) treatment were analyzed by a new HBsAgGi ELISA system and compared with other HBV markers (qHBsAg, HBV-DNA, and HBcrAg). To characterize target particles of HBsAgGi, immunoprecipitated (IP) particles by HBsAgGi antibody were analyzed by reverse-transcription PCR for quantifying HBV-RNA.

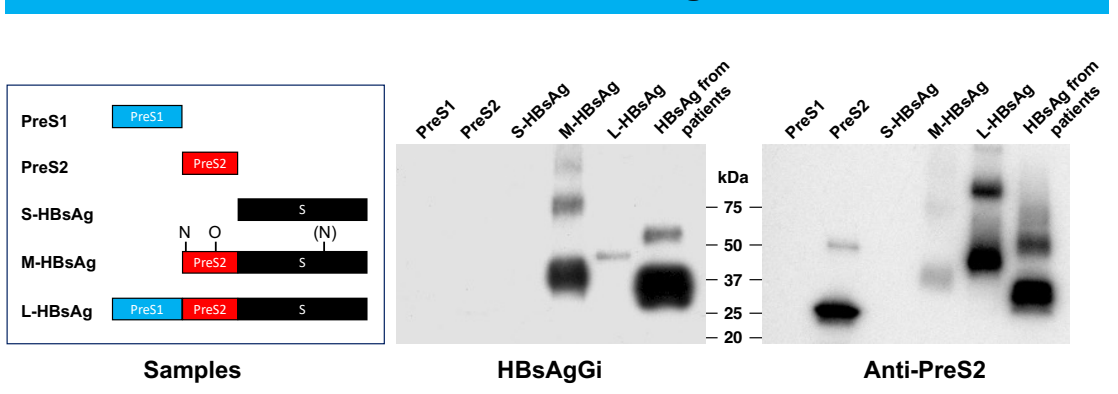
HBsAgGi is specific to genotype C and present in ER to Golgi in M-HBsAg-expressing cells



Distribution of HBsAgGi, HBV-DNA, qHBsAg and HBcrAg at baseline in NA naïve EPH and ENH patients



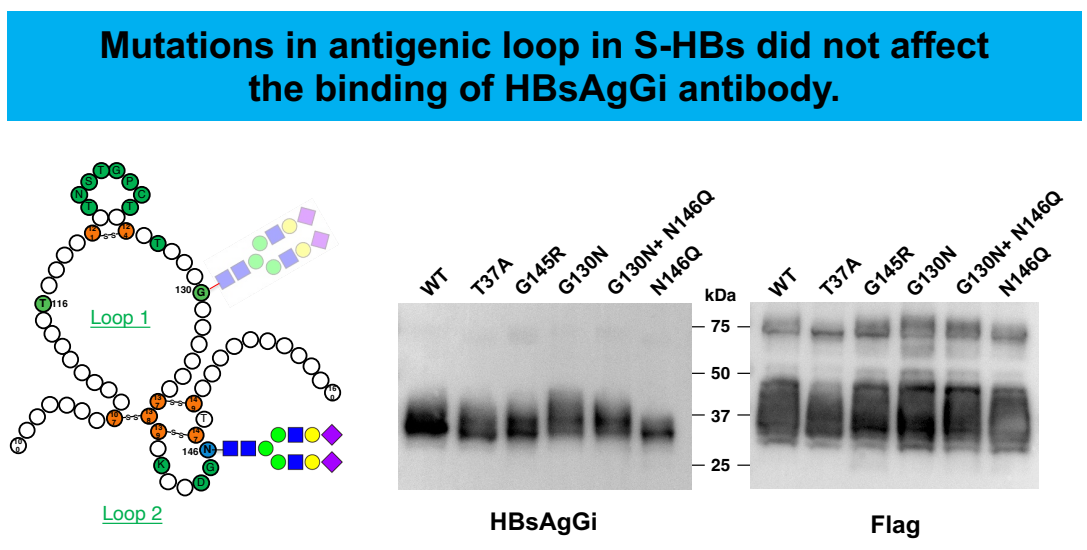
HBsAgGi antibody specifically recognizes M-HBsAg but not L-HBsAg



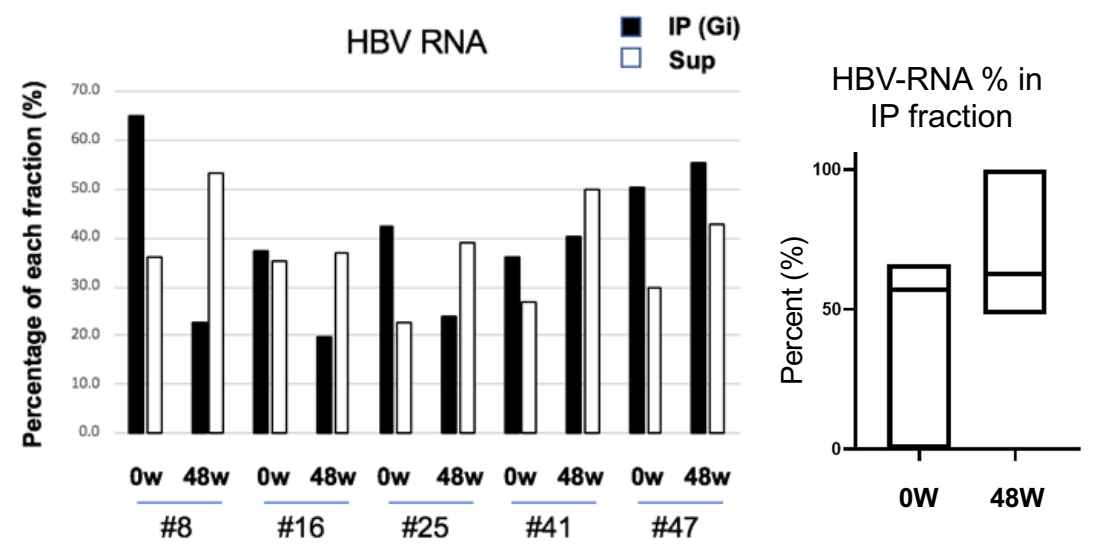
Correlation of HBsAgGi with HBV-DNA at baseline in NA naïve CHB patients

HBsAgGi vs HBV DNA

Mutations in antigenic loop in S-Hbs did not affect the binding of HBsAgGi antibody.



HBV particles immunoprecipitated by HBsAgGi antibody contain HBV-RNA.



CONCLUSIONS

- HBsAgGi antibody recognizes HBV particles in genotype specific manner dependent on the presence of *O*-glycans in PreS2 domain.
- HBsAgGi levels were significantly correlated with the HBV-DNA level in CHB patients during therapy.
- HBsAgGi-positive virions contain HBV-RNA.
- Thus, HBsAgGi specifically presents in infectious fraction of HBV virions containing HBV-DNA or HBV-RNA.