論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

Hepatitis B virus (HBV) infection is clinically diagnosed by the detection of viral surface protein (HBsAg) using commercial immunoassays whereas recombinant HBsAg produced in yeast cells has been used for prophylaxis. The antigenic and the immunogenic properties are shifting due to mutations in the HBsAg amino acid sequence. Recent studies reported that several diagnostic and vaccine escaping HBV have been circulating among patients, which demands the development of a novel diagnostic and vaccination strategies. In this study, three wild type HBsAg (adr4, W1S and W3S [subtype adr]) isolated from clinically infected patient and nineteen synthetic single/double/multiple amino acid substituted mutants have been cloned and expressed in HEK-293T cells, confirmed by Western blot and immunofluorescence assay. The antigenic properties/reactivity of all wild-type and mutants using commercial ELISA kits demonstrated that lysine (K120) and aspartate (D123) simultaneously affected the W3S HBsAg antigenicity and thereby led to diagnostic failure. Moreover, single mutation at P120K, T123D, N146G and combined mutation at Q129R/G145R significantly reduced the antigenic reactivity of W1S HBsAg. The commercial ELISA based antigenic reactivity of HBsAg also strongly correlated with the results of predicted Ai alterations of affected amino acids due to the specific mutation. These findings will be very helpful for developing new diagnostic assays and designing novel vaccine strategies against HBV infection.

A very peculiar HBs escape mutant was found and the mechanism why the mutant escaped from some laboratory HBs detection kits was well elucidated. This finding will lead to improve HBV infection diagnostics and the vaccine design as mentioned above. And this mutant has another characteristic feature that is highly glycosylated. It is very interesting to analyze whether the HBV is more infectious or not.

Thus, this work deserves to receive the Ph. D.

論　文　内　容　の　要　旨
Synopsis of Thesis

氏　名 Name	MD. GOLZAR HOSSAIN
論文題名 Title	Investigation of a Novel Hepatitis B Virus Surface Antigen (HBsAg) Escape Mutant Affecting Immunogenicity (免疫原性に影響を及ぼす新規B型肝炎ウイルス表面抗原（HBsAg）変異体の検討)

論文内容の要旨

〔目　的 (Purpose)〕

Mutation in the hepatitis B virus surface antigen (HBsAg) may affect the efficiency of diagnostic immunoassays or success of vaccinations using HBsAg. Thus, this study is aimed to investigate the antigenicity and immunogenicity of a mutated and diagnostic escape HBsAg.

〔方法ならびに成績 (Methods/Results)〕

Here, the *in vitro* antigenicity of three wild-type HBsAg open reading frames (ORFs) (adr4, W1S [subtype adr] and W3S [subtype adr]) isolated from clinically infected patients and nineteen synthesized single/double/multiple amino acid-substituted mutants were tested with commercial ELISA kits. Immunofluorescence staining of transfected cells and Western blot analysis confirmed that these ORFs were expressed at comparable levels in HEK-293 cells. W1S and adr4 were clearly detected, whereas W3S could not be detected. Using the same commercial immunoassay kit, we found that the single mutants, K120P and D123T, were marginally reactive, whereas W3S-aW1S and the double mutant, K120P/D123T, exhibited antigenicity roughly equivalent to the wild-type wako1S. On the other hand, the single mutants of W1S, P120K and T123D, significantly impaired the reactivity, while W1S-aW3S and the double mutant of W1S, P120K/T123D, resulted in a complete loss of antigenicity. In addition, ELISA revealed reduced HBs antigenicity of two mutants, W1S N146G and W1S Q129R/G145R. These commercial ELISA-based antigenic reactivities of HBsAg were also strongly correlated with the predicted *Ai* alterations, which predicts structural conformation, of affected amino acids due to the specific mutation.

〔総　括 (Conclusion)〕

This study showed for the first time that lysine (K120) and aspartate (D123) simultaneously affected HBsAg antigenicity, leading to diagnostic failure. These findings will improve diagnostic assays and vaccine development.