論　文　内　容　要　旨

CTL-associated and NK cell-associated immune responses induce different HBV DNA reduction patterns in chronic hepatitis B patients

（B 型肝炎患者における細胞障害性 T 細胞、NK 細胞関連肝炎による異なった免疫応答と HBV DNA の変化について）

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Background and Objectives:

HBV is a member of the Hepadnaviridae, a family of small hepatotropic DNA viruses. The virion consists of an envelope containing three related surface proteins (S,M,L-HBs proteins) and a nucleocapsid that is constituted by the core protein. Many variants of HBV genome have already been reported and it is well known that PreS region in S gene is a hot spot where genomic deletions sometimes occur.

PreS deletion variants has been shown to be associated with the clinical status, the immune response to interferon, or HBV replication. However, the association between the presence of HBV deletion variants and activation of hepatitis has not been fully clarified.

At the clinical aspects, while HBV chronically infected with human hepatocytes, hepatitis occurs by the repetitive activation of host immunity, and it could be one of the most important causes of liver disease progression. However, it is difficult to predict when the clinical events will be occurred, such as ALT elevation or HCC development.

In the present study, we analyzed the association between the immune response and changes in the proportion of Pre-S deletion variants.

Materials and Methods:

First, we performed sequence analysis using HBV patients sera and determined the most frequent deletion in PreS region. (nt3016 ~ nt3198) To establish a quantifying system of the Pre-S deleted HBV, we designed new specific primer sets which can amplify only Pre-S deleted HBV genome and PreS deleted HBV plasmid. We measured both wild type HBV (HBV-wt) DNA levels and Pre-S deleted HBV DNA levels by real time PCR in sera obtained from HBV-infected mice and chronic hepatitis B patients, and we compared changes of ALT and proportions of Pre-S deleted HBV DNA levels.

Results:

At first, we selected 4 chronic HBV patients who could be followed up for more than 3 years. The first patient is a 32 years old man. At 3 years after the first visit, an ALT flare occurred. During this ALT flare, the proportion of Pre-S deleted HBV, increased transiently. However, the proportion subsequently recovered to the similar level before the ALT flare. The second patient is a 26 years old man. During the follow-up 7 years, ALT flares occurred 6 times. The proportions of Pre-S deleted HBV, increased along with each ALT flare except one, and at the last ALT flare, the proportion of Pre-S deleted HBV reached to nearly one hundred percent.

The third patient is a 32 years old woman. ALT flare occurred twice within 3 years, and

the proportion of Pre-S deleted HBV, was increased with each ALT flare. However, following the flares, the proportion of Pre-S deleted HBV, rapidly decreased to pre-flare levels. The fourth patient is a 30 years old man. Although his HBV DNA level was high, his ALT remained steady for 3 years. 3 years after his first visit, ALT flare occurred. But the proportion of Pre-S deleted HBV did not increase.

To analyze the difference, we used two severe hepatitis mouse models. In our previous studies, we identified two different immune reactions (Cytotoxic T Lymphocyte: CTL-mediated hepatitis or Natural Killer : NK cell-mediated hepatitis) that may occur in the severe hepatitis B mouse model. HBV infected human hepatocyte chimeric mice were prepared and human PBMC was injected. After PBMC injection, severe hepatitis was occurred in the mouse livers. In these 2 mouse models, different immune responses were occurred in the mouse livers. So, we measured the proportions of Pre-S deleted HBV using these mouse models.

In HBV infected mice without antiviral treatment, the proportion of Pre-S deleted HBV remained unchanged. However, in CTL-mediated hepatitis mice, the proportion of Pre-S deleted HBV increased. On the other hand, in mice with severe NK-cell mediated hepatitis, the proportion of Pre-S deleted HBV did not significantly change after inoculation of PBMCs. We also analyzed using HBV-infected chimeric mice with entecavir treatment. DNA Levels of both HBV-wt and Pre-S deleted HBV decreased together, and the proportion of Pre-S deleted HBV remained steady.

As the result, we considered that the increase of Pre-S deleted HBV was induced by CTL-mediated immune response.

To verify this association between the immune response and changes in the Pre-S deleted HBV proportion, we measured the Pre-S deleted HBV proportions in 59 Japanese patients who infected with HBV genotype C and have never been treated with antivirals. The number of patients in which Pre-S deleted HBV proportions were greater than 5% was significantly higher in chronic hepatitis B patients than in asymptomatic carriers (P=0.023).

Recently, several target epitopes to HBV-specific CTLs have been identified in the Pre-S region. Increases in the proportion of Pre-S deleted HBV may represent a specific response to the activation of CTLs.

Conclusion:

we identified the association between immunological responses in chronic hepatitis B patients and the presence of preS1 deleted HBV variants. These results may help to distinguish chronic hepatitis patients from chronic HBV carriers with normal ALT levels.