Basic research for the development of hepatitis C vaccine

（C 型肝炎ワクチン開発に向けた基盤研究）

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Background

Hepatitis C is a liver disease caused by hepatitis C virus (HCV). HCV can cause chronic hepatitis infection with 80% rate of HCV infected individuals. Chronic hepatitis can lead to cirrhosis and hepatocellular carcinoma. HCV carriers are approximately 185 million people worldwide. The number is still increasing especially in developing countries. Currently HCV treatment can cure approximately 90% of HCV patients but prophylactic and therapeutic vaccines have not yet been available. The development of hepatitis C vaccines is necessary because HCV drugs are extremely expensive, not accessible to all HCV patients.

In this point of view, I aimed to develop feasible hepatitis C vaccines as a final goal. First of all, I evaluated safety and efficacy of a novel hepatitis C vaccine candidate which was composed of inactivated whole-virion prepared from cell culture-generated HCV (HCVcc) and established by my collaborators; Dr. Kato and his colleagues in Department of Virology II, National Institute of Infectious Disease. Then to evaluate the prophylactic and therapeutic effects of hepatitis C vaccine candidates, I hypothesized that chimeric virus constructed by HCV and GB virus B (GBV-B) and New World monkeys (NWMs) can be an appropriate animal model. GBV-B is closely related to HCV and can cause chronic hepatitis in NWMs. Furthermore, to improve replication efficiency and virulence in the animal model, I analyzed that the differences of humoral/cellular immune responses in acute and chronic phases of GBV-B infection. To apply these novel findings to the animal model, I sought to decline the cellular immunity by depleting cytotoxic T lymphocytes ($CD8^+$ T cells) in marmosets in collaboration with Dr. Yoshida.

Materials and Methods

I injected the novel hepatitis C vaccine candidate together with adjuvant (Alum or K3-SPG) into common marmosets (*Callithrix jacchus*). And I evaluated safety of the vaccine candidates. The expression of *IFNγ* mRNA was evaluated after cultivation with HCV core peptides by real-time PCR. Then a novel HCV/GBV-B chimera constructed by HCV and GBV-B was inoculated into a red-handed tamarin (*Saguinus midas*). In acute and chronic phases of GBV-B-infected NWMs, anti-E2, core and NS3 antibody titers were evaluated by ELISA. In addition, I compared the rate of NK cells expressing cytotoxic markers ($CD3^- CD16^+ CD56^{dim} CD335^+ CD159a^+$) in NWMs with acute and chronic phases of GBV-B infection by flow cytometry. Depletion of $CD8^+$ T cells was performed by administration of a novel marmoset CD8-specific monoclonal antibody (mAb). I observed the periodical kinetics of $CD4^+$ T cells, $CD8^+$ T

cells, B cells and NK cells subsets of lymphocytes.

Results

By immunization with HCVcc with K3-SPG, the expression of *IFNγ* mRNA in splenocytes was increased after cultivation with HCV core peptides. Vaccination-related abnormality was not detected. Together with the data in terms of antibody responses provided by the collaborators, I concluded that the combination of HCVcc and K3-SPG induced humoral and cellular immune responses and showed the possibility to become a safe and effective hepatitis C vaccine.

After inoculation of the HCV/GBV-B chimera, it was observed that the chimera persistently replicated in a tamarin for more than two years after the intrahepatic inoculation, although the viral load was relatively low. This result will help to establish the novel animal model for developing hepatitis C vaccines on the basis of the HCV/GBV-B chimera in the major framework of HCV genome.

In order to seek factors for higher replication efficiency and virulence, I analyzed that the differences of humoral/cellular immune responses in acute and chronic phases of GBV-B infection. The induction of anti-E2, core and NS3 antibodies in chronically infected monkeys was much delayed and it took up to 1-3 years to reach plateau antibody titers after GBV-B inoculation. Furthermore, cytotoxic NK cells increased after viremia in the GBV-B-infected monkey with acute clearance. On the other hand, the rate of cytotoxic NK cells in the chronically infected monkey was low as much as that in naïve and the GBV-B cured-monkeys. These results suggest that the induction of antibodies and cytotoxicity of NK cells may be associated with the clearance of GBV-B infection.

To apply these findings to an animal model, I sought to deplete $CD8^+$ T cells in marmosets by administrating novel marmoset CD8-specific mAb. It was observed that drastic and specific depletion of $CD8^+$ T cell subset. It is hoped that this method will contribute to establish an animal model which shows higher replication efficiency and virulence of the HCV/GBV-B chimera.

Discussion

In conclusion, to establish an animal model for evaluating prophylactic and therapeutic effects of hepatitis C vaccine candidates, I generated the HCV/GBV-B chimera and inoculated it into a tamarin. The chimera showed persistent replication *in vivo*, which implies to be an appropriate animal model. Furthermore, I clarified that the induction of antibodies and cytotoxicity of NK cells are one of the factors to persist GBV-B infection. Applying these findings, I established the method to decline cellular immunity in NWMs by administrating novel marmoset CD8-specific mAb for improving the animal model.

I believe that these knowledges found in my study are newly and critical for HCV vaccine study and will contribute to effectuate development of hepatitis C vaccines in the future and lead to a breakthrough in hepatitis C study.