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学位論文内容の要旨
（Summary of dissertation）

博士の専攻分野の名称　博士（医　学）　氏名　リョン　チェン　リン
（Degree conferred: Doctor of Philosophy）　（Name of recipient: LEONG CHEAN RING）

New insight into the host and virus interaction of HBV
（HBV に対する宿主応答の解析に関する研究）

【Background and Objectives】

Hepatitis B is a major health burdens worldwide considering that more than 240 million people are chronically infected. The mechanisms by which Hepatitis B virus (HBV) established and maintains as a chronic infection are poorly understood. Although adults acquired HBV is generally cleared by a robust immune response, approximately 5% of individuals are remains unresolved during the adulthood infection. It is generally believed that HBV is a "stealth" virus that avoids the host innate immune responses, but there is increasing evidence that activation of innate host cell signaling pathways plays a major role in limiting the HBV infection in the immuno-competent adults. Hence, we believe that the innate immune response to HBV infection is driven by an early response to specific "pathogen-associated molecular pattern" and subsequently drives the adaptive immune response that is crucial for viral clearance. In the current work, we would like clarify which of the viral components and related pathways that trigger the innate immune response against HBV. Furthermore, we also intended to shed light on the effector of innate immune system that limits the propagation of HBV.

【Materials and Methods】

Though HBV has so far to be reported exclusively infects hepatocytes, the liver is an immunological organ with non-parenchymal cells (Kupffer cells, sinusoidal endothelial cells) and parenchymal cells (hepatocytes and epithelial cells) with unique immunological characteristics, it is crucial to examine the HBV infection with a new insight into the host and virus interaction. Herein, we employed a liver-specific *in vivo* transfection method to investigate the immunological events in the genetically modified knock out mice. The hydrodynamic injection allow us to deliver the replication competent HBV full genome into the mice livers and inspect the consequence of lacking certain molecules or pathways to the pathogenesis of the virus. We also examined a range of candidate genes for prominent potential to suppress the HBV replication by co-expressing them with the HBV replicative full genome. The HBV nucleic acid (RNA and DNA) as well as the protein (antigen) was analyzed as marker of the HBV propagation.

【Results & Discussion】

We found that the immune-competent wild type mice presented a self-limiting acute infection with complete elimination of the viral template DNA, so as the mice lacking the comprehensive RNA sensing pathway (*MAVS*$^{-/-}$ and *TICAM-1*$^{-/-}$). On the other hand, impair of the molecules essential to the IFN induction pathway (*Irf-3/7*$^{-/-}$ and *Ifnar*$^{-/-}$) leads to a greatly elevated virus titers in the KO mice even though the intrahepatic DNA template was cleared. Interestingly, dysfunction of the adaptive immune effector like T cells and B cells (*Rag2*$^{-/-}$) leads to a persistency of the viral over a long period of time. Our attention has also been called to the fact that lacking the MyD88 adaptor

molecule which activates the TLR9 pathway causes the inability of the viral clearance. These results indicated that the IFN induction is necessary to contain the propagation of HBV at the early stage while the RNA sensing pathways did not seems to play an indispensable role in such context. The *in vitro* study using the immortalized hepatocytes or hepatoma cell lines also suggests that lacking in the DNA sensing pathway (cGAS or STING) could be responsible for the absence of IFN induction in these cell lines.

Following the outcome on the importance of type I IFN in suppressing the HBV replication, we screened a number of interferon inducible genes that responsible to impede HBV replication. ISG20, an interferon inducible 3' to 5' exonuclease has been demonstrate with the capability to suppress the HBV DNA, RNA and antigen production in the hepatocytes derived cells. Over all, our study has provided the evidence that IFN and IFN-inducible molecule (ISG20) are crucial in limiting the HBV infection, but not in the case of the viral DNA clearance. Thus, the missing link between induction of type I IFN and anti-HBV cellular effectors require further investigation.

【Conclusion】

Toll-Like Receptors (TLRs) and cytoplasmic RNA sensors have been reported to be involved in the regulation of Hepatitis B virus (HBV) replication but remain controversial due to the lack of a natural infectious model. Our current study sets out to characterize aspects of the role of the innate immune system in eliminating HBV using hydrodynamic based injection of HBV replicative plasmid and knockout mice deficient in specific pathways of the innate system. The evidence indicated that viral replication was not affected by MAVS or TICAM-1 knockout, but absence of IRF3 and IRF7 transcription factors, as well as the interferon (IFN) receptor, had an adverse effect on the inhibition of HBV replication, demonstrated the dispensability of MAVS and TICAM-1 pathways in the early innate response against HBV. Besides, we demonstrated that ISG20 is a crucial host restriction factor that limits HBV replication and propagation. Mechanistic studies of the inhibitory effect of ISG20 on HBV replication have crucial implications on the better understanding of virus-host interaction as well as the viral pathogenesis during HBV infection.