Studies on coronaviruses causing enteric infections in domestic animals in Japan

（日本における家畜の下痢原因コロナウイルス
に関する研究）

Summary of Doctoral Thesis

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Summary

Coronaviruses (CoVs) cause a wide range of diseases in farm and domestic animals, some of which are a threat to the farming industry and has serious effect on the economy. Economically important CoVs of farm animals include porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), bovine torovirus (BToV) and bovine coronavirus (BCoV), which result in enteric infection causing diarrhea in young animals. In 2013, a huge porcine epidemic diarrhea (PED) outbreak has occurred in Japan after a period of 7 years without any outbreak, which causes high morbidity and mortality in young piglets. On the other hand, BToV causes mild to moderate diarrhea in claves. BToV with the full length hemagglutinin-esterase (HE) gene was always isolated from diarrheal feces; however, the viruses lost the HE protein as a result of mutation of the HE gene following several passages in cultured cells. These findings suggest that the HE protein is important for replication and pathogenesis in animals, but is not essential for replication in cell culture. In the present study, phylogenetic and antigenic characterization of newly isolated PEDV in Japan is described. On the other hand, biological characterization of the HE protein of BToV focusing on anti-interferon (IFN) activity is examined.

1. Phylogenetic and antigenic characterization of newly isolated PEDV in Japan

PEDV causes severe diarrhea and dehydration, which usually lead to high morbidity and mortality, especially in piglets. Though vaccination program has been performed in pig farms throughout the whole Japan, PED has re-emerged. In the first chapter, we describe the isolation of new PEDV strains from recent outbreaks in Japan and the phylogenetic relatedness of the newly isolated strains with PEDVs circulating worldwide. Furthermore,

we attempted to reveal the antigenic relationship among the new strains and vaccine strain. To evaluate the mechanism by which a large outbreak of PED occurred in Japan, where the majority of sows are vaccinated, we isolated two new strains of PEDV from the intestines of piglets and found that they are more similar to US isolates (group II PEDV) than to the Japanese vaccine strain (group I PEDV). We compared the antigenicity of the vaccine type strain and newly isolated strains by means of a neutralization test using sera from a number of pigs from various farms; the results revealed that they are antigenically similar. This is the first report of the similarity of group I and II viruses using sera from individual pigs vaccinated with group I virus. These data suggest that the large outbreak of PED in Japan may not be attributed to inefficient vaccination but may be due to the extremely high virulence of the newly appearing viruses. It was also suggested in the literature that the poor biosecurity is one of the major causes of huge PED outbreak worldwide.

2. Biological characterization of the HE protein of BToV

BToV, which causes diarrhea in calves, contains the HE protein on the viral envelope when isolated from the host, although HE is often lost from the virion after multiple passages in cultured cells. This suggests that HE protein may be important for replication or pathogenesis in infected animals, but is not indispensable for the replication in cultured cells. In the second chapter, we explored the biological functions of the HE protein. We isolated the BToV Niigata-3 (Nig-3) from diarrheal specimen of the cattle. Nig-3-3 with HE (HE+) from the viruses with 3rd passage level and Nig-3-8 without HE (HE-) at 8th passage level were obtained by cloning using human rectal tumor (HRT-18) cells. The mutation has arisen in the HE gene of Nig-3, which makes a stop codon at the

amino acid number 109 of HE gene. We cloned Nig-3-3 and Nig-3-8 using HRT-18 cells and compared their growth in cultured cells. Nig-3-8 (HE-) grew more efficiently than Nig-3-3 (HE+), suggesting the possibility that HE inhibits BToV growth in cultured cells. Human embryonic kidney 293T (HEK 293T) cells expressing the HE protein also suppressed the infection of the Sindbis virus. These results suggest that HE protein is an obstacle for the growth of various viruses in cultured cells.

3. Anti-IFN activity of HE protein of BToV

In the previous chapter, it was revealed that HE protein is not essential for the replication but is an obstacle for virus replication in cultured cells. Although the HE protein may hinder viral growth in cultured cells, the HE protein likely has an important biological function for viral growth in animals and possibly for viral pathogenesis in the host, since specimens isolated from diseased cattle contain the virus with the HE protein. In this chapter, we explored the anti-IFN activity of the HE protein of BToV using IFN-α and IFN-β on the growth of HE+ and HE- viruses. IFN-α depressed the growth of HE- viruses, but not that of HE+ ones, whereas IFN-β has no influence on their growth. HE protein expressed in HEK 293T cells were examined whether it acts as IFN-α antagonist by using Sindbis virus. The infection of Sindbis virus in HEK 293T cells expressing HE protein was not affected by the IFN-α treatment, though the infection was depressed in cells expressing the truncated HE protein after treatment with IFN-α. This indicated that HE protein acts as an IFN-α antagonist. These results collectively suggest that HE plays an important role in the pathogenesis in BToV infections as an IFN antagonist to innate immunity.

As studied above, vaccination with group II virus strains as well as good biosecurity could assist to protect pigs from large outbreaks of PED. On the other hand, we found that HE protein suppresses BToV infection in cultured cells. More importantly, we showed that HE functions as an IFN antagonist, which indicates that HE is an important pathogenic factor for the infection in animals. The studies on two different CoVs causing enteric diseases shown in this thesis will contribute to the development of anti-viral strategies as well as delineation of the pathogenic mechanisms of the CoV infection.