論 文 の 内 容 の 要 旨

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論文題目　**Studies on Murine Norovirus Infection in Mice and Its Influence on Animal Experiments**

(マウスのマウスノロウイルス感染症及びその動物実験への影響に関する研究)

Background

Human noroviruses (HNV) are a member of the Caliciviridae family, major cause of non-bacterial gastroenteritis worldwide. HNVs cause 23 million cases of gastroenteritis, 50,000 hospitalization and 300 deaths annually in United States alone. All ages are susceptible to infection and it spreads rapidly in semi-closed communities such as hospitals, cruise ships, college campus and military bases. In infected individuals, complaints appear to be severe nausea, vomiting, and watery diarrhea within 12-24 hours, and majority of infected persons recover in 1 to 3 days. Although norovirus gastroenteritis is generally mild and self-limited short duration, infection can be severe and fatal in immune compromised and vulnerable individuals. Despite the significant economic loss and impact of public health caused by HNV, there is no efficient treatment and vaccine due to the lack of a cell culture system and small animal model.

To date, the majority of study HNV infection relies on challenging with virus to human volunteers. In experimentally infection with HNV, 11 of 16 persons showed clinical gastroenteritis (watery diarrhea and/or vomiting) with lasting of systematic illness 1-2 days and shedding virus for up to 56 days.

Histological analysis of proximal intestinal biopsy specimens from ill volunteers demonstrate an intact intestinal mucosa with specific histological changes, including broadening and blunting of the villi, shortening of the microvilli, enlarged and pale mitochondria, increased cytoplasmic vacuolization, and intercellular edema. Although human volunteer studies provide materials for determine property of HNV pathogenesis, but it is still circumscribed to understand aspect of the virus. The limitations of human as a model system are i) experimentation is difficult and expensive, ii) sampling is restricted and technically demanding, iii) bio-containment requirements for the live and highly infectious virus, iv) ability to study live virus is reduced by the lack of a tissue culture system.

These difficulties associated with in vitro cultivation and human volunteer study collectively demonstrate the requirement for small animal models which is facilitated the study and lead to greater understanding of HNV.

In 2003, mouse norovirus (MNV) is discovered in immunocompromised mice lacking the recombination-activating gene 2 (RAG2) and signal transducer and activator of transcription 1 (STAT1) (RAG2/STAT1-/-). MNV-1 demonstrated to infect and replicate in murine macrophage cell lines and cultured primary dendritic cells and macrophages from STAT1-/- mice. Severely innate immune deficient mice were showed systemic disease and lethality with encephalitis, cerebral vasculitis, meningitis, hepatitis and pneumonia after infection. But adaptive immune system deficient mice, lacking T and B cells response, (RAG1-/- and RAG2-/- mice) are resistant to MNV-1 induced lethality. And Rag-/- mice showed high levels of viral RNA in lung, liver, spleen, proximal intestines, brain and feces while wild-type (129 and C57BL/6) cleared MNV-1 infection. These studies suggested that adaptive immunity is not necessary to protection against lethal MNV infection but are required to prevent the dissemination and continuous replication of MNV.

MNV-1 is closely related to HNV (genogroup V norovirus – HNV is in I, II and IV) and shares numerous biochemical and genetic features, including fecal-oral transmission, replication in the intestine, and fecal shedding, with HNV [16]. And a natural host is relatively cost-effective and genetically well-characterized. These facts lead MNV to excellent model of HNV research.

MNV is most prevalent in research mice. Recent serologic study to detect antibodies against MNV in serum of laboratory mice from multiple research institutions in North America demonstrated 22.1% presence of MNV antibody in evaluated 12,639 serum samples. Furthermore 76 feces specimens collected from various breeding colonies in Berlin and Germany showed 63% of samples contained MNV RNA by one-tube real-time RT-PCR method. MNV infection typically show no visible sign and clinical symptoms of disease but persist with shedding virus in feces for more than 8 weeks in immunocompetent mice [20], while MNV induce lethal infections in only severely innate immune deficient mice, such as STAT1-/- and interferon$\alpha\beta\gamma$R-/-. MNV is stable upon exposure to pH extremes (pH 2 to 10) and 56 degrees, remains infectious for 7 days at room temperature in fecal material. These aspects of MNV may lead to high prevalence and difficulty of eradication of MNV in mouse facilities.

This prevalence of MNV in mouse facilities may lead to unreliable and irreproducible results and impact immune response to infection with other viruses. Because both circulating macrophage and dendritic cells

serve as reservoir for MNV result in inducing a robust type I IFN response in 129/SvJ mice [24] and . This issue was continually discussed and Cadwell et al reported that CR6 MNV strain, but not MNV1, induce striking abnormalities in Paneth cell function by interaction with a mutation in the Crohn's disease susceptibility gene Atg16L1. And Karen et al demonstrated that MNV4 alters antigen presenting activity of dendritic cells result in aggravates disease progression in a mouse model of inflammatory bowel disease [26]. In one report, MNV-G led to mouse parvovirus DNA levels higher in mesenteric lymph node, spleen, and small intestines and also virus shedding longer in BALB/c mice. These reports demonstrate that MNV infection may impact on biomedical research significantly.

Chapter 1

Mouse norovirus S7 (MNV-S7) was isolated in Japan 2007 and known to most prevalent pathogen of laboratory mice. Although serologic importance of MNV-S7 in mouse research colonies, pathology of MNV-S7 in mouse was not clearly understood. To assess histopathological changes from infection of MNV-S7, C57BL/6, interferon gamma knockout ($IFN\gamma^{-/-}$) and interferon regulator factor 3 and 7 double knockout ($IRF3/7^{-/-}$) mouse were inoculated perorally, and major organ were analyzed. Although all infected mice were seroconverted to MNV, mice showed no clinical symptoms and gross lesions except that only $IRF3/7^{-/-}$ mice showed mild intestinal inflammation which is related to edema, shorten vilus and activation of peyer's pathes in distal colon. Viral antigens of MNV-S7 were detected in the mesenteric lymph node of $IFN\gamma^{-/-}$ and $IRF3/7^{-/-}$ mice, epithelium and lamina propria of $IRF3/7^{-/-}$ mice by immunohistochemistry. Especially, mast cells in MLN of $IFN\gamma^{-/-}$ were presented proliferation and matched to immunoreactive cells by probing MNV specific antibody. These data reveal possibility that MNV-S7 has identical pathogenicity with MNV-1 but tropism for mast cells.

Chapter 2

Murine norovirus (MNV) is known to be the most prevalent virus in laboratory mice colonies in the world. Although it generally induces no clinical symptoms in immunocompetent mice, some studies showed that MNV alter disease progression of inflammatory bowel disease in mice. However, it has not been reported that MNV significantly impacted on another viral infection. In the present study, I examined whether or not MNV infection influence on the progression of mouse hepatitis virus (MHV) infection, which is also an important infectious disease in mice. MNV-S7 and MHV-A59 were propagated using RAW 267 and DBT cells, respectively. Female, 7-weeks-old C57BL/6J mice were inoculated with 2,000 PFU of MHV-A59. A part of the mice had been inoculated with MNV-S7 per orally before one week of MHV infection. Viral growth of MHV in the liver was determined by plaque assay and the histopathological changes were examined. No significant difference was observed between mice infected MHV-A59 infection alone and those coinfected MNV-S7 and MHV-A59 at 3 days postinfection. However, the growth of MHV in mice coinfected with MNV was lower than those infected MHV alone. In addition,

the inflammatory lesions in the liver of mice coinfected with MNV were lighter than those infected MHV alone. IFNα production was not changed but MHV specific neutralizing antibody was amplified by previous MNV infection. This is the first report that MNV infection alters the progression of viral infection in mice.

Chapter 3

Human norovirus (HNV) cause more than 95% of gastroenteritis worldwide. Although it is realized to important in public health, there is no vaccine or drug because hampered study by the lack of a small animal model and unable culture *in vitro*. Various challenge of replicating HNV *in vitro* was unsuccessful in yielding but only mouse norovirus 1 reported that replicate in RAW 264.7 cells and has tropism for macrophage and dendritic cell. HNV infection induces gastroenteritis which is similar symptoms to food allergy as watery diarrhea due to fail of result in an imbalance in ion exchange and/or water transfer. But interaction between norovirus and mast cell, which play an important role in allergic response, was not established. The present study reports that MNV-S7 inoculated IC-2 cell (and P-815), mast cell line, showed increase of viral titer and immunofluorescent by probing MNV-S7 specific antibody. These data suggest that MNV-S7 infect in mast cell, and aberration of mast cell is responsible for the diarrhea of MNV-S7.