論　文　の　内　容　の　要　旨

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Pseudotyped vesicular stomatitis virus for analysis of entry of arenaviruses and its application to serodiagnosis of Argentine hemorrhagic fever

(水疱性口内炎ウイルスのシュードタイプを用いたアレナウイルスの細胞侵入過程の解析とアルゼンチン出血熱の血清診断法への応用)

Viral hemorrhagic fever (VHF) is a serious illness characterized by extensive vascular damage and bleeding diathesis, fever, and multiple organ dysfunction. Infection of variety of viruses results in similar clinical manifestation of VHF, however, mode of transmission, reservoir animals and clinical outcome including fatality rate in humans are different among VHFs.

These viruses causing VHF are distributed throughout four virus families, the *Arenaviridae*, *Bunyaviridae*, *Filoviridae*, and *Flaviviridae*. Several of these viruses cause severe VHF with high morbidity and mortality and can be highly infectious by

aerosol dissemination, promoting serious concern about weaponization of these pathogens. Amongst these pathogens, Lassa virus and South American hemorrhagic fever virus, which consists of 5 virus species, are arenaviruses.

Currently, there are no specific treatments approved for use against arenavirus hemorrhagic fevers. Present disease management consists of general supportive care; monitoring and correcting fluid, electrolyte and osmotic imbalances and treating hemorrhage with clotting factor or platelet replacement. Convalescent immune serum therapy may be effective in treating cases of VHFs caused by Junin and Machupo virus infection, but the availability of such serum is extremely limited.

The only arenaviral hemorrhagic fever for which studies have been undertaken toward development of a vaccine has been Argentine hemorrhagic fever (AHF) caused by Junin virus. A live-attenuated vaccine, called Candid #1, has been evaluated in controlled trials among agricultural workers in AHF-endemic areas, where it appeared to reduce the number of reported AHF cases with no serious side effects. It is not known if the Candid #1 vaccine would be effective against other South American hemorrhagic fevers. In addition, this vaccine is available only in Argentine.

As these viruses are categorized into BSL4 pathogens in many countries thus can be handled only in specific institutions with BSL4 facilities, analysis of pathogenesis of these pathogenic arenaviruses, development of therapeutic agents and vaccines for arenaviral hemorrhagic fevers have been impeded. In addition, epidemic areas of these diseases are mainly located in developing countries, that is, Sub-Saharan Africa and South America. Pharmaceutical companies have not been given their prior attention to develop medicines for these infectious diseases because of the profits generated from them.

To solve these problems, in Chapter 1 of this study, I have developed vesicular stomatitis virus (VSV) pseudotypes bearing Lassa virus (LASV) envelope protein (LASpv) and Junin virus (JUNV) envelope protein (JUNpv), respectively. LASpv and JUNpv generated in several mammalian cell lines exhibited high infectivity in various mammalian cell lines. Arenaviral envelope proteins on the VSV pseudotypes were glycosylated by high-mannose type oligosaccharide. Endosomal low pH-induced endocytosis of VSV pseudotypes was confirmed by the use of lysosomotropic agents. Low pH induced membrane fusion by Lassa and Junin envelope protein was monitored by syncytium formation and reporter gene activities. The infection of JUNpv to Huh7 cells was mediated by binding to human transferrin receptor 1 (hTfR1) as a receptor. Involvement of cathepsin L in JUNpv cell entry was suggested. These results found in Chapter 1 indicate that the VSV pseudotypes developed in this study can be used to study arenavirus envelope proteins with respect to the biological functions including receptor interaction in the entry process. In addition, a gene encoding the envelope protein of JUNV or LASV is not encoded in VSV gene. Thus the VSV pseudotypes do not produce infectious progeny virus, so that they can be safely handled in a BSL2 containment level.

In Chapter 2 of this study, I have developed a neutralization (NT) assay using a novel VSV pseudotype bearing JUNV glycoprotein (GP) and the NT has been shown to be equally sensitive and specific in detection of neutralizing antibodies compared to NT using infectious JUNV. Cross-reactivities of antibodies in AHF patients' sera with Machupo, Guanarito, Sabia and Chapare viruses were also analyzed by the NTs using VSV pseudotypes bearing the respective viral GPs, IgG-ELISAs using the respective viral nucleoproteins (NPs) and indirect immunofluorescence assays (IFAs) using the

respective viral NP expressing cells. AHF patients' sera have been shown to cross-react to other clade B arenaviruses in IgG-ELISA and IFA. However, there was no cross-neutralization at all to other arenaviruses causing South American hemorrhagic fever in the NT using VSV pseudotype, indicating the NT is useful in differential diagnosis among South American hemorrhagic fever.

Through these studies, I have developed safe research tools for arenaviruses, and useful diagnosis methods for AHF. These tools and diagnosis methods can be used in almost all research institutes all over the world at BSL2 or lower level. I think the present study could contribute the research and development of treatment and prevention methods for arenavirus HF.