Graduate School of Infectious Diseases

学位論文内容の要旨
Abstract of the dissertation

博士の専攻分野の名称：博士（感染症学）　氏名：ボニフェイス　ポンゴンボ　ロンベ
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学位論文題名
Study on serological diagnostic assays for Crimean-Congo hemorrhagic fever
（クリミア・コンゴ出血熱の血清診断法に関する研究）

Crimean-Congo hemorrhagic fever (CCHF) is an important tick-borne zoonotic disease with a wide geographic distribution. It is caused by the CCHF virus (CCHFV) belonging to the genus *Orthonairovirus*, family *Nairoviridae*, order *Bunyavirales*. CCHFV infection of humans is potentially associated with fatal hemorrhagic fever. Serological surveillance of CCHFV infection of animals serves as an indicator of public health risks, and early detection of human infection is important for prevention of viral spread. However, CCHFV infection is not serologically monitored due to the limited availability of diagnostic tools. Existing serological diagnostics are human-specific and not readily available in CCHFV-affected areas. Handling live infectious CCHFVs requires a high biocontainment facility, limiting the capacity of serological diagnosis such as neutralization tests. Hence, there is a need to improve serological assays for CCHFV surveillance and epidemiology studies to prevent potential outbreaks.

In Chapter I, a simple procedure for expression and purification of the recombinant CCHFV nucleoprotein (NP) was established, and its utility as an antigen for ELISA for the detection of CCHFV-specific IgG in serum/plasma of humans and animals was evaluated. cDNA of the NP open reading frame was cloned into a mammalian expression plasmid and introduced into cultured human cells. The expressed NP molecule was purified from the cell lysate using cesium-chloride gradient centrifugation and used as an antigen for several ELISA protocols. In addition to its animal species-independent utility, the established CCHFV NP-based ELISA showed abilities comparative to a commercialized ELISA kit for the detection of CCHFV NP-specific human IgG. These results demonstrate the usefulness of the CCHFV NP-based ELISA established in this study for seroepidemiological studies.

In Chapter II, antigenic regions on the NP molecule were investigated. Using recombinant chimeric NPs between CCHFV and Nairobi sheep disease virus (NSDV), which is another nairovirus, it was found that the amino acids at positions 240-482 might include dominant epitopes recognized by anti-CCHFV IgG antibodies. In

contrast, IgG antibodies to NSDV reacted to the region consisting of amino acid positions 1-240. Accordingly, all of the CCHFV NP-specific monoclonal antibodies generated in this study recognized this antigenic region. ELISA with a series of synthetic peptides based on the CCHFV NP sequence revealed that IgG antibodies in CCHFV-infected monkeys and patients were reactive to some of the peptide antigens. Fine epitope mapping of CCHFV NP is expected to improve the virus-specific serological assays for surveillance of CCHFV infection of humans and animals.

This study focused on serological diagnostic assays for CCHFV infection. The findings not only provide useful information to improve the sensitivity and specificity of serological tests for CCHF but also help to enhance surveillance systems of CCHFV infection of both animals and humans through the development of rapid detection tests such as an immunochromatography-based diagnostic assay. These may contribute to CCHF prevention and control by reducing animal-to-human and human-to-human transmission of the virus.