Summary of Doctoral Dissertation

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Title of Doctoral Dissertation

Inhibiting Canine Distemper Virus infection by generating monoclonal antibodies targeting Hemagglutinin Protein

(ヘマグルチニンタンパク質を標的としたモノクローナル抗体による犬ジステンパーウイルスの感染阻害)

Recently, the number CDV hosts has been increasing. With the recent report in cases of monkey infections, it has been argued that CDV might evolve to infect humans. In this study, we developed and characterised neutralising antibodies which target the H protein of CDV. CDV is a member of the Paramyxoviridae family to which the Morbillivirus genus belongs. This genus also includes other viruses such as Rinderpest virus and Measles virus (MeV). MeV is thought to have evolved from rinderpest virus and crossed over from cattle to human. This is synonymous with what has been observed with CDV, which has demonstrated its ability to cross the species barrier. Hemagglutinin (H) protein is the main target for neutralizing antibodies. Neutralizing antibodies can target (1) H protein – receptor interaction, (2) H protein – fusion protein interaction or (3) block the conformational change of fusion protein.

Firstly, H protein head domain of CYN07-dV strain of CDV was produced by culturing in HEK293T mammalian cells. The supernatant from the cell culture was then purified by Nickel affinity and size exclusion chromatography which showed that the expressed CDV H protein existed as dimer under physiological conditions.

The purified CDV H protein was then immunized into 8-week-old female Wistar rats to generate lymphocytes which were fused with myeloma to produce hybridoma cells. Screening of the hybridoma samples revealed 6 monoclonal antibodies (mAbs) with affinity for CDV H protein.

Fusion inhibition assay was then done to determine the neutralising ability of the mAbs. The results indicate that the mAbs had differing inhibition ability. ELISA analysis showed that some mAbs were able to cross-react with the H protein of 6 CDV strains (Kyoto vaccine strain, 5VD, A71/75, CYN07-dV, MSA5 and Ac96I strains) and MeV's IC-B95 and Edmonston strains. In addition, all antibodies inhibited the interaction between the SLAM receptor and CDV H protein. The data, therefore, strongly suggests that some mAbs likely interact with residues conserved in all the 6 CDV and 2 MeV strains located near the receptor binding site.

Finally, the study has shown that monoclonal antibodies able to inhibit CDV infection by binding to the H protein were developed. These antibodies will further help us understand CDV infection mechanism to fight CDV infection.