



HOKKAIDO UNIVERSITY

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Abstract of Doctoral Dissertation

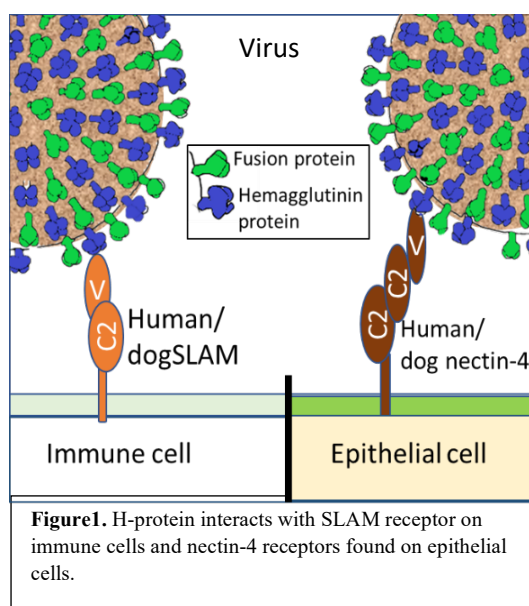
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Title of Doctoral Dissertation

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)

Introduction



Recently, the number CDV hosts has been increasing. With the recent report of monkey infections¹, it has been argued that CDV might evolve to infect humans². 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 barrier¹. 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

Results and Discussion

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

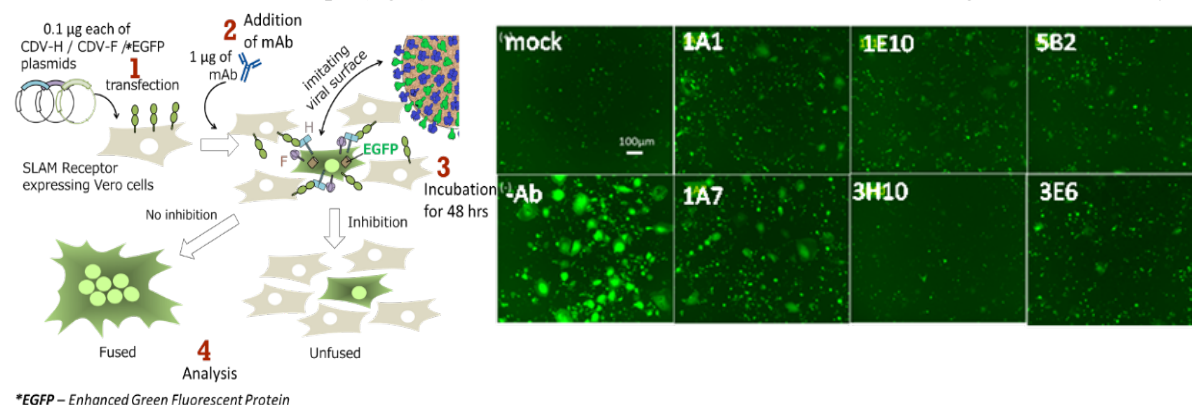
exclusion chromatography which showed that the expressed CDV H protein existed as dimer under physiological conditions.

Summary of ELISA results

Strain mAb	CDV strains						MeV	
	A75/17	5VD	MSA5	CYN07dV	KYOTO	Ac96I	ICH	Edmonston
1A1	+++	+++	+++	+++	+++	+++	++	+++
1A7	+	+	+	+	+	+	-	+
1E10	+++	+++	+++	+++	+++	+++	-	-
3E6	+++	+++	+++	+++	+++	+++	++	+++
3H10	+++	+++	+++	+++	+++	+++	++	+++
5B2	+++	+++	+++	+++	+++	+++	-	+++

The purified CDV H protein was then immunized into 8-week-old female Wistar rats. After 14 days, lymphocytes were isolated from the rats' iliac lymph nodes and fused with X63-Ag8.653 myeloma to produce hybridoma cells. Screening of the hybridoma samples revealed 6 monoclonal antibodies (mAbs) with binding affinity for CDV H protein. After ELISA analysis it was found that some of the mAbs were cross-reactive for both CDV and MeV strains and results are summarised in the table.

Fusion inhibition assay was then done to determine the neutralising ability of the mAbs. The principle of this assay is illustrated below (left). Final images were taken after 48 hours and analysis done using BZ-X710 fluorescence microscope (right). The results showed that the mAbs had differing inhibition ability



with 3H10 mAb having the highest rate of inhibition compared with the rest of the antibodies while 1A7 mAb inhibition fusion inhibition ability was low. The mock had no H protein gene transfected thereby highlighting the importance of H protein for cell fusion and, consequently, syncytia formation.

Amino acid sequence comparison of the 6 CDV strains (Kyoto vaccine strain, 5VD, A71/75, CYN07-dV, MSA5 and Ac96I strains) and MeV ICH and Edmonston strains reveals high conservation of residues near the receptor binding site. In addition, all antibodies inhibited the interaction between the SLAM receptor and CDV H protein. The data, therefore, strongly suggests the mAbs such as 1A1, 3E6 and 3H10 mAbs likely interact with residues conserved in all the 6 CDV and 2 MeV strains located near the receptor binding site.

References

1. Sakai, K. *et al.* **Lethal canine distemper virus outbreak in cynomolgus monkeys in Japan in 2008.** *J. Virol.* **87**, 1105–1114 (2013).
2. Sakai, K. *et al.* **Canine Distemper Virus Associated with a Lethal Outbreak in Monkeys Readily Adapted to Use Human Receptors.** *J. Virol.* **87**, 7170–7175 (2013).