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# INFORMATION

Hokkaido University granted the Doctor of Veterinary Medical Science Degree to the following researcher on September 30, 1996, under the regulation (1962) authorizing the granting of the Doctor's degree to qualified researchers who was not graduate of the Graduate School of Veterinary Medicine.

The title of the thesis and other information are as follows :

## BASIC STUDIES FOR THE CONSTRUCTION OF A RETROVIRAL VECTOR SYSTEM TO TRANSFER VECTOR GENES INTO THE CENTRAL NERVOUS SYSTEM

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Retroviral vector systems have become important tools with which to efficiently transfer genes into mammalian cells. Because of its stable gene expression with little toxicity and antigenicity, retroviral vector systems is a most convenient means of transferring genes of interest into the central nervous system (CNS). However, retroviral packaging cell lines have been constructed with non-neurophilic retroviruses, resulting in less efficient gene transfer into the CNS. The neurophilic murine leukemia virus (MLV), FrC6 and its molecular clone A8, proliferates in the CNS of rats *in vivo* as well as in rat glial cells *in vitro*. Therefore, it is considered that a novel retroviral vector system based on FrC6 virus would improve pseudo-viral infectivity in the CNS. One of major determinants of A8 viral neurophilicity was included in its *env* gene by producing recombinant viruses between A8 and non-neurophilic MLV. In this study, the biological efficiency of the envelope protein of FrC6 virus was investigated to construct a novel retroviral vector system that could effectively introduce foreign genes into the CNS.

At first, to characterize the envelope protein of FrC6 virus, monoclonal antibodies (MAbs) recognizing this virus were established. Thirty MAbs, each of which reacted with the Friend MLV-producing cell line, were tested for potential neutralizing activities. Only two of them inhibited proliferation of the A8 virus. These two MAbs were ineffective or had very weak neutralizing activities toward the non-neurophilic Friend MLV clone 57. Further characterization of the MAbs by immunoprecipitation revealed that four recognized the envelope protein of A8 virus. Two of these four recognized the surface glycoprotein gp70, requiring a conformational epitope for this

recognition, while the other two, which were reactive with the transmembrane protein p15E, were conformation-independent. Both MAbs against gp70 distinguished neurophilic and non-neurophilic viruses to some extent, through neutralizing activity or binding activity detected by immunoprecipitation, whereas the two MAbs against p15E reacted with the viruses in a similar manner. Furthermore, one of the MAbs distinguished the viral antigen in the walls of the vacuoles that compose the spongiotic lesion induced by FrC6 viral infection of the brain.

To characterize the biological efficiency of the envelope protein of FrC6 virus, a phenotypically mixed pseudo-virus carrying the envelope protein of FrC6 was constructed. The retroviral pseudo-virus carrying the envelope proteins of A8 virus and Moloney MLV was produced by transfecting the *env* gene of A8 virus (A8*env*) into the Moloney MLV based packaging cell, $\psi$CRE. The phenotypically mixed pseudo-virus infected rat glial cell lines as well as NIH 3T3 cells, whereas the pure pseudo-virus produced in $\psi$CRE without A8*env* expression, infected the glial cells less efficiently. Furthermore, $\psi$CRE expressing A8*env* produced a higher titer of pseudo-virus than normal $\psi$CRE cells. The ability of the phenotypically mixed pseudo-virus to infect the glial cells was abolished by a neutralizing antibody against A8 virus, which did not reduce that of the pure pseudo-virus produced in $\psi$CRE to infect NIH3T3 cells. These results indicated that the envelope protein of A8 is assembled into pseudo-viral particles and that it contributes to glial cell infection by this virus.

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