学 位 論 文 内 容 の 要 旨
Abstract of Doctoral Dissertation

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学 位 論 文 題 名
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Characterization and single chain Fv construction of neutralizing antibody to measles virus

（麻疹ウイルスに対する中和抗体の機能評価と一本鎖 Fv 断片の構築）

Measles Virus (MV) is a major cause of childhood morbidity and mortality worldwide although an effective vaccine is available. The MV infection is initiated by binding of cellular receptors on the target host cells to hemagglutinin protein of MV (MV-H), a surface glycoprotein responsible for the target cell entry. Signaling lymphocyte activation molecule (SLAM) and nectin-4 are identified as cellular receptors. The binding triggers membrane fusion between the virus envelope and the host cell plasma membrane, mediated by the fusion (F) protein. Thus both glycoproteins, especially H protein, become neutralizing targets. Nowadays, several neutralizing antibodies to MV have been developed against different antigens so far, and most of them target to H protein. Our collaborators have previously prepared the neutralizing mouse monoclonal antibody (MAb) 2F4 using a cell line expressing the H protein as an antigen. The infection assays demonstrated that 2F4 MAb shows high neutralizing titersand the epitope of MAb 2F4 is overlapped with the binding site of the receptors although the epitopes have not been fully determined yet. The single chain variable antibody fragment (scFv) strategy is one of the most popular methods in antibody engineering because of its lower immunogenicity, and its small molecular size allowing better tissue penetration. The aim of this study is to develop scFvs of MV neutralizing antibodies and reveal their molecular basis for the inhibition of MV entry.

For this purpose, I constructed the expression plasmid of scFv from MAb 2F4 (2F4-scFv). The scFv was expressed in *E. coli*, refolded and purified for characterization. By using the recombinant 2F4-scFv protein, I performed surface plasmon resonance (SPR) experiment including binding kinetics to the antigen MV-H head domain. The results showed that the scFv specifically binds to the MV-H at μM level of K_D value, which is lower than those of Fab form and extracellular domains of cellular receptors, SLAM and nectin-4. Further competitive analysis demonstrated that the 2F4-scFv is able to inhibit the binding of MV-H to its cellular receptors. Conventional fusion assay was also carried out using receptor-expressing cells, showing that the 2F4-scFv effectively inhibits the syncytia formations mediated by the interaction between MV-H and cellular receptors. Collectively, the 2F4-scFv is able to inhibit competitively the binding of MV-H and cellular receptors, thereby to inhibit the membrane fusion. I expect that the newly prepared 2F4-scFv will enable us to further investigate the inhibitory mechanism of cell entry via MV-H, thereby to design an effective vaccine and anti-viral drug against MV infection.