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主　論　文　の　要　旨

論文題目　Study on Immunological Detection of Swine Influenza Virus Using Rabbit Monoclonal Antibody
（ウサギモノクローナル抗体を用いたSwine Influenza Virusの免疫検出に関する研究）

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論　文　内　容　の　要　旨

Sandwich enzyme-linked immunosorbent assay (Sandwich-ELISA) is one of the standard diagnostic tests for pathogenic disease in human and animals. It can detect a target antigen in clude samples such as saliva, mucous, urine or other secretion. In commercial Sandwich-ELISA kit, immobilized and detector antibody are mostly obtained from monoclonal and polyclonal antibody (mAb and pAb), respectively. However, the use of pAb tends to increase cross-reactivity in the test kit, and makes difficult to reproduce the test kit after use-up of the qualified serum. Thereby, the use of dual mAb or recombinant Ab is desirable in sandwich-ELISA. In this research, swine influenza virus (SIV) is selected to be antigen because the infection of SIV is an important disease in swine industry that causes a huge economic loss to livestock industry and can transmit into human. The early SIV detection is significantly important to protect a viral outbreak in pig and human. The objective of this study is, therefore, to develop a rapid immunoassay test for SIV detection by sandwich-ELISA using rabbit mAb, because rabbits are supposed to make highly specific antibody than mouse. Ecobody technology is used to generate rabbit mAb, because the entire process of selecting B cells, RT reaction of light chain and heavy chain of antibody, DNA amplification and expression by cell-free protein synthesis will finish within two days.

Firstly, swine influenza virus (SIV) vaccine was immunized to a rabbit

for producing antigen-specific B cells. Then single B cells bound to SIV magnetic beads from this rabbit were isolated by using a cell micropicker and the immunoglobulin mRNA of each cells was individually amplified by RT-PCR and PCR for inserting the amplified DNA into expression vectors with T7 promoter and T7 terminator. Finally, the expression construct for light chain and heavy chain of Fab were amplified by PCR for cell-free protein synthesis (CFPS). The binding activity of the Fab proteins thus synthesized were measured by Enzyme-linked Immunosorbent Assay (ELISA). Totally, 55 Fabs were synthesized by CFPS and all of them had a higher signal to SIV than to negative control. It indicated that all of the rabbit Fab generated from single B cells bound SIV vaccine specifically.

Sandwich ELISA requires two antibodies as coated antibody and detector antibody to capture antigen by the two antibodies in a sandwich manner. Therefore, we prepared Fabs with two different peptide tags, 6xHis-tag and PA-tag, for the same Fab genes. Optimal combinations of coated Fab and detector Fab for immunoassay test were investigated by mAb sandwich ELISA. As detector mAb, Fab-PA showed a higher signal than Fab-6xHis. Therefore, Fab-6xHis and Fab-PA were used as coated Ab and detector Ab, respectively. Four Fab-6xHis and four Fab-PA were coupled for the selection of optimal pairs by sandwich-ELISA. Four pairs of Fab-6xHis and Fab-PA presented the high biding activity. In addition, these pairs were examined for the minimal detection of SIV by sandwich-ELISA. One of the pair showing the highest OD value in the sandwich ELISA were selected for the optimization of the ELISA. The Fabs of this pair was purified to increase the sensitivity of the assay. Eventually only 0.5 ng SIV vaccine was successfully detected by mAb sandwich-ELISA with the selected Fab pair. This limited detection of SIV concentration was close to the previous report that used mAb against nucleoprotein and pAb labelled with HRP as immobilized and detector antibody, respectively. It indicated that mAb sandwich-ELISA by two Fab with different tags can detect SIV with high sensitivity. In summary, Fab generated by Ecobody technology can be applied for the generation of immunodetection kit in a high-throughput manner.