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KANCHALA UTID: STUDIES ON THE SUSCEPTIBILITY OF VARIOUS CELL LINES IN SUPPORTING GROWTH OF JAPANESE ENCEPHALITIS VIRUSES. THESIS ADVISORS: CHANPHEN WIWAT, Ph.D., SOMSAK PANTUWATANA, Ph.D., PRANEE SITTHISARN, M.Sc. 135 P. ISBN 974-662-682-5

Japanese encephalitis (JE) is one of the serious public health problems in Southeast Asia, including Thailand, because of its high fatality rate and its expansion into new areas. Both inactivated and live attenuated JE vaccines have proved to be effective to control JE viruses in many countries. Inactivated JE vaccine that is made from mouse brain is widely used in Thailand. However, this vaccine contains high rates of foreign proteins that are associated with special risk categories and the national supply is insufficient. Attempts have been made to find an appropriate cell line for development as substrate for the mass production of JE viruses.

Fifteen continuous cell lines from various origins were chosen for studying the susceptibility to JE viruses and the ability to support the growth of three strains of JE viruses. JE viruses could produce plaques in all of the selected cell lines except the 3T3-cell. The growth of JE viruses was assayed by plaque method. It was found that 3 cell lines, Vero, BHK-21 and PS clone D, produced high titers of viruses about 10^8 , 10^7 and 10^7 PFU/ml, respectively. All 3 strains of JE viruses produced very clear plaques that could be detected in 3 days in BHK-21 cell line; thus BHK-21 cell line was used as a substrate in the plaque assay. The three selected cell lines were infected with three strains of JE viruses that were adjusted to make multiplicity of infection (M.O.I) at 0.1, 1 and 10. The highest amount of viruses was obtained when JE viruses strain Beijing was grown in Vero cell line. The virus titers were increased to 8.04 and 8.1 \log_{10} PFU/ml in 48 hours post-infection when the inoculum sizes were adjusted to 0.1 and 10 M.O.I., respectively. The inoculum size of JE virus strain Beijing adjusted at 0.1 M.O.I. was selected for use in microcarrier bead technique.

The microcarrier beads were prepared by derivatization of Sephadex G50 with DEAE. It was found that these microcarrier beads could support the attachment and growth of three cell lines on their surface. The microcarrier beads gave the highest cell densities when Vero cells were used with the growth ratio of 14.9 in comparison with the others at the same inoculum size, 1.0×10^5 cells/ml, in 8 days post-inoculation. BHK-21 cells seemed to be easier to detach from the bead surfaces. After Vero cells were cultured and reached confluent monolayer, the inoculum of JE viruses' strain Beijing was adjusted to 0.1 M.O.I. and supplied to the culture. The highest virus titers were increased to 8.89 \log_{10} PFU/ml at 4 days post-infection. It was found that the highest yield of viruses could be obtained when the microcarrier beads cultures were used. The results show that it is possible to develop cell culture technique using microcarrier beads system for the multiplication of JE virus to manufacture vaccines in the future.