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MULTIPLICATION OF AN ATTENUATED HOG CHOLERA VIRUS, LPC-CHINA STRAIN IN VARIOUS CELL CULTURES

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Primary swine testicle (ST) cell was the most susceptible cell to propagate the LPC-China strain (LPC) HC vaccine virus. The virus titer was reached up to $10^{7.0}$ $TCID_{50}$/ml and found not increasing significantly throughout 9 passages. Primary swine kidney (SK) and PK-15 cells were also capable of growing the virus, but were less sensitive than primary ST cells. Interestingly, primary rabbit kidney cell and RK-13 cell line were insensitive to grow the rabbit adapted HC virus. The vero cell, a green monkey kidney cell line, also failed to propagate the virus. The homo virus interference test (HVIF) and fluorescent antibody cell culture technique-2 step method (FACCT-2 step) were equal sensitive to detect the virus.

Various cell culture system derived from swine tissues have been used for primary isolations and for fundamental studies of HC virus (1,2,3). The tissues mostly used included kidney, spleen, bone marrow, testicle, lymph-nodes and leucocytes. Replication of the virus was also achieved on bovine kidney cells (4), and chicken fibroblast (5). Both virulent and attenuated HC virus were cultivated successfully in cell cultures (6). HC vaccine produced by growing attenuated strain of HC virus was also reported (7,8). The LPC strain of HC virus attenuated by serial passage in rabbits for more than 800 passages has been widely used for HC control in Taiwan (9). However, difficulty in quality control was the disadvantage of vaccine produced from infected tissues. Multiplication of LPC virus in tissue culture of swine origin has been reported (10). The LPC virus was non-cytopathic effect (CPE) to the susceptible cells. Therefore the HVIF (10, 11) and FACCT-2 step techniques (12) were currently used for detecting the LPC virus. The purpose of this study was to seek a suitable cell for growing the LPC virus, which may be used for tissue culture vaccine priduction.

MATERIALS AND METHODS

Viruses; LPC-strain HC virus, 816 rabbit passage, was used for the multiplication test. Western equine encephalomyelitis (WEE) virus grown in swine testicle cell was used for challenge in the HVIF. GPE⁻ virus, an attenuated HC vaccine virus obtained from the National Institute of Animal Health (NIAH) of Japan, was also grown in ST cell and used in the HVIF.

Cells: Primary ST and SK cells were prepared by trypsinization of testes and kidneys obtained from 6-8 weeks old healthy pigs. Primary RK cells was prepared by trypsinization of kidneys obtained from healthy rabbits. weighted 1-2 kgs. The techniques used for trypsinization of tissues were previously described (12). PK-15, MVPK of swine origin and vero cell lines were also used for growing the LPC virus.

Multiplication of LPC virus in various types of cells: Confluent monolayers of each type of cells growing in the 3-liter roller bottles were used for virus multiplica-

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tion. Eight ml of 10x tissue emulsion of the LPC virus infected rabbit spleen containing 10^6 rabbit infective dose (RID) per ml were used as the initial inoculum for each bottle. After 60 minitues' absorption at 37°C, the cell sheet was rinsed with PBS and refilled with 50 ml of Eagle's minimum essential medium (MEM) containing 2% fetal calf serum and incubated for 5 days. After incubation, the cultured fluid was harvested and the cell sheet was frozen and thawed for three times. The mixtures of culture fluid and cell debris were used as the inoculum for further passages. Totally six passages were made for each type of cells.

Homo virus interference test (HVIF): The HVIF used for titration of LPC virus was previously described by Lin and Lai (12).

Fluorescent cell culture technique-2 step method (FACCT-2 step): The FACCT-2 step method described previously (12) was used for titration of LPC virus. The virus was grown in the primary ST cells for 5 days, then the virus fluid was titrated by transferring to PK-15 cell and stained with HC fluorescent antibody conjugate. Growth

Growth curve of LPC virus, ST6 passage: The sixth passage of LPC virus in ST cell (ST6) was used for the growth curve study. The growth curve was determined in ST cells by titrating the mixture of cell associated and cell-free virus using the HVIF and FACCT-2 step techniques. A series of 6 wells plastic plates of ST cell monolayers were infected with LPC virus, ST6 passage at a multiplicity of 10 $TCID_{50}$/cell. After 60 minitues' absorption at 37°C, the well were rinsed three times with warm PBS and refilled with 2 ml of MEM containing 2% fetal calf serum. For viral growth curve, at 1 day interval, 6 wells of infected cells and fluid were removed, and frozen and thawed three times. Then the cell debris were removed by centrifugation. The supernatant was used for virus titration.

RESULTS

The virus titers of serial passage of LPC virus in various types of cells was shown in Figure 1. The primary ST cell was the most susceptible cell to propagate the LPC virus. The virus titer of the first and the second passages were $10^{6.5}$ $TCID_{50}$/ml. The virus fluid in the third passage had a titer of 0.5 Log higher than that in the previous passages. However, in the further passages, the virus titer did not increase significantly. The primary Sk cell was also capable of growing the virus. In the first three passages, the virus titers were found considerable low, not exceeding $10^{2.5}$ $TCID_{50}$/ml. However, a rapid increase in virus multiplication was found in the 4th passage, having a titer of $10^{5.0}$ $TCID_{50}$/ml. The virus titers in the 5th and the 6th passages did not show any significant changes as oompared to that of the 4th passage. The two SK cell lines, PK-15 and MVPK cells were found poor in virus multiplication despite increasing passages. Approximately, a titer of $10^{2.5}$ $TCID_{50}$ /ml was detected throughout six passages. Interestingly, primary rabbit kidney cell and RK-13 cell line, were not capable of growing the rabbit adapted HC virus. The vero cell, a green monkey kidney cell line, also failed to propagate the virus.

The growth curve of LPC virus, ST6 passage was shown in Figure 2. The infectivity of the virus was detected as early as one day postinoculation, with a titer of 1.5 Log $TCID_{50}$/ml. Multiplication of the LPC virus was slow. The highest infectivity of the virus, 7.6 Log $TCID_{50}$/ml, was observed on day 5 postinoculation. Then the virus declined gradually. The HVIF and FACCT-2 step techniques were equal sensitive to detect the LPC virus.

DISCUSSION

The susceptible cell types for growing the LPC virus were similar to those reported by Liu (10). The most susceptible cell was primary ST cell. The virus titer of the first passage was $10^{6.5}$ $TCID_{50}$/ml. However, there was no significant increase throughout

9 passages. Interestingly,rabbit kindney cell was insensitive to grow the highly rabbit adapted virus. Studies on the virus distribution of LPC virus in rabbit also indicated that kidney tissues had very low or no virus infectivity (10).

The results suggest that 3 to 5 tissue culture passages of the LPC virus using primary ST cell derived from SPF pigs can be effectively applied to produced HC tissue culture vaccine that may keep its antigenicity of rabbit origin.

For the *in-vitro* assay method, the FACCT-2 step technique is the better one for the detection of LPC virus in terms of the shorter period requirement as compared to the equal sensitive technique, HVIF test, that required 13 days for the completion of the test.

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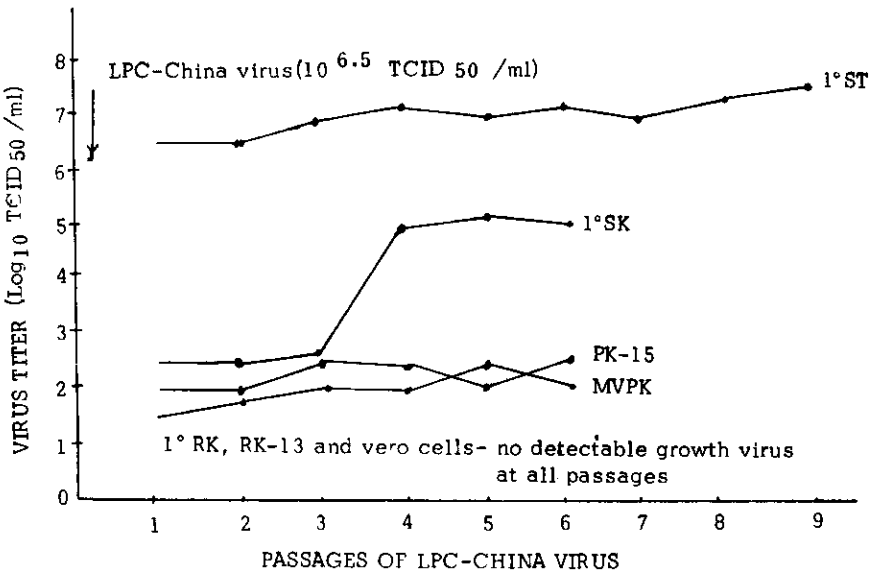

Fig. 1 Virus titers of an attenuated HC virus, LPC-China strain multiplied in different cells at different passages.

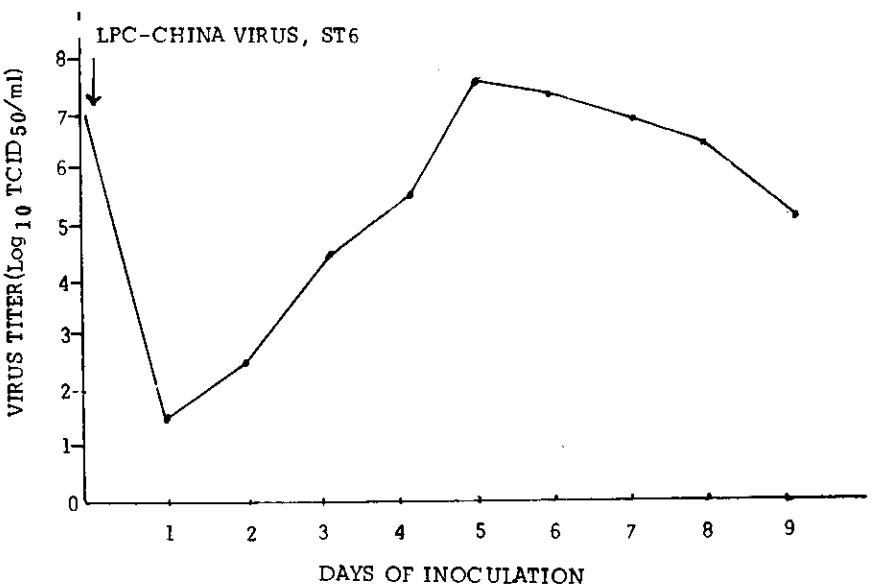

Fig. 2. Growth curve of LPC-China virus, the 6th passage in primary ST cell

兔化豬瘟疫苗毒之組織細胞感受性試驗

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初代豬睪丸細胞增殖兔化豬瘟毒最爲敏感，病毒力價可達到 $10^{7.0}$ $TCID_{50}$/ml，但在連續九代繼代後病毒力價並無顯著的增高。初代豬腎細胞及豬腎株細胞（PK－15）亦可增殖兔化豬瘟毒，但其敏感度則較初代豬睪丸細胞差，初代兔腎細胞及兔腎株細胞（PK－13）無法殖增以兔子減毒的兔化豬瘟毒。其他細胞如猴腎細胞株（Vero）亦無法增殖兔化豬瘟毒。

干擾法及螢光抗體二段法對測定兔化豬瘟毒的敏感度一樣。