Sensitivity of Cells to Poliovirus

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The detection of every poliovirus in the stool is most important at the final stage of the polio-eradication program. We examined stool specimens collected from acute flaccid paralysis patients in the Western Pacific Region in the past 8 years. No wild poliovirus has been isolated since March 1997 to this date. At this stage, sensitive poliovirus detection is crucial.

In the past we introduced La cells (mouse L cells expressing human poliovirus receptor) (1) for the isolation of poliovirus from specimens contaminated by other enteroviruses. Subsequently, WHO, which first recommended RD and HEp-2 cells, recommended using L20B (another L cell line expressing human poliovirus receptor) (2) cells in place of HEp-2 cells. Thus, we needed to know whether L20B was actually better than La for virus isolation or not.

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The growth medium for RD, HEp-2, and L20B cells was Eagle’s MEM supplemented with 10% fetal bovine serum (FBS) and 0.15% bicarbonate. For La, the growth medium was Dulbecco’s MEM supplemented with 10% FBS and 0.15% bicarbonate; for maintenance of the cells the concentration of FBS was reduced to 2%.

RD, HEp-2, L20B, and La cells were cultured in 24-well plates containing 1 ml/well of the medium. The virus samples, Sabin type 1, type 2 and type 3, were diluted ten-fold serially, and each dilution was inoculated into four wells (100 μl/well). We examined the cultures at 36°C for cytopathic effect (CPE) every day and calculated the titer.

Development of CPE was the slowest in La for all the virus strains, while that in L20B was similar to that in HEp-2 and RD (Fig. 1, left side). Part of the infected cells were harvested either when CPE appeared or on day 7 if CPE did not appear. The freeze-thawed whole culture lysates were inoculated to the respective cells in a volume of 100 μl and examined for the induction of CPE. The titers thus obtained were the same for all the cell lines (Fig. 1, right side). It was concluded that La had the same susceptibility to poliovirus as L20B, but that the development of CPE was much faster in L20B than in La. We support the present recommendation of L20B by WHO.

REFERENCES


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