Experimental Variation in Mouse Virulence of Echo 9 Virus. (25199)

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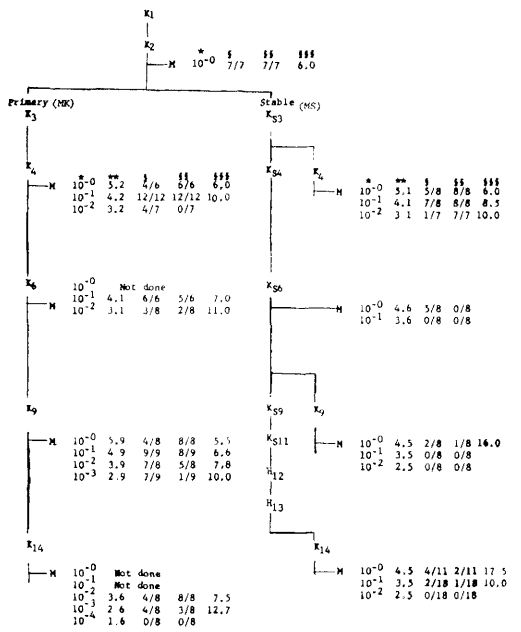
As reported previously mouse virulence of type III poliovirus (mouse-adapted) grown in tissue culture (TC) depended on the host cell and its maintenance medium. When grown in monolayer TC in Medium 199, monkey (Rhesus) kidney (MK) cells enhanced mouse virulence while HeLa cells tended to inhibit it(1). Many other serial cell lines including monkey stable (MS) kidney cells, possessed the same inhibitory property(2). The present study was conducted to see whether mouse virulence of Echo 9 virus would be influenced by the host cell in a similar way.

Materials and methods were similar to those described previously(1) except for the following modifications. Echo 9 virus, Bourn strain (3) was serially passed in monkey (Rhesus) kidney tissue culture (MKTC), plaque purified once, and again transferred to MKTC by Drs. J. L. Melnick and Y. Kanda. This virus (Bourn LR $451K_8$ P_1 K_1) was used and was designated as K_1. The K_1 virus was again transferred to MKTC (K_2, Chart 1), and from which 2 lines of serial MKTC passages were carried, (a) in primary cells and (b) in MS cells(5), all fed with Eagle's medium with 2% calf serum. For initiating the serial passage, 0.2 ml of the undiluted TC fluid was inoculated to each of 4 TC tubes, each containing 1.5 ml medium. At the end of the experiment, as MS cells were not available, HeLa cells were used instead for the last 2 passages. The virus from the primary cells was harvested usually on the 4th or 5th day when most of the cells were destroyed while that from MS cells on the 8th or 9th day as cytopathic effect was greatly delayed in the MS cells. At various passage levels the virus was titrated in TC as well as assayed in mice. Titration in TC was made by inoculating 0.2 ml of each of serial 10-fold dilutions into primary MKTC in Medium 199 with 2% calf serum, using 6 tubes per dilution. Assay of virulence was made in 1-day-old Swiss mice by intramuscular inoculation of 0.02 ml of different dilutions, using 1 or 2 litters of mice per dilution each litter consisting from 5 to 9 mice. As titer of virus from the MS cells was relatively low, the virus was often transferred to primary cells in Eagle's medium with 2% calf serum for 1 passage in an attempt to raise its titer before virulence assay, (K_9, K_{14}, Chart 1).

Results. These are summarized in Chart 1. Before the experimental passages the virus was found virulent as all 7 mice inoculated with undiluted (not titrated) TC fluid died in 6 days (average). After one passage the viruses (K_4) grown in either primary or MS cells were all virulent for the suckling mice. After further passages, however, the virus grown in primary cells remained consistently virulent as tested at passage level K_6, K_9, and K_{14} while the virus grown in MS cells and tested at comparable passage levels lost most of its virulence as indicated by paralysis rate, death rate and survival time of those mice which eventually died. Although titer of virus from MS cells was relatively lower, the difference in virulence between the 2 lines was not due to the quantity of virus in the inoculum. For instance, at passage level K_{14}, a dose of $10^{3.6}$ $TCID_{50}$ virus from primary cells killed all 8 inoculated mice in 7.5 days (average) while a bigger dose ($10^{4.5}$ $TCID_{50}$) of virus from MS cells killed only 2 out of 11 mice in 17.5 days (average). (In the later group, 4 out of 11 mice became paralyzed but 2 of them recovered.)

Discussion. These experiments demonstrated clearly that the mouse virulence of Echo 9 virus could be definitely influenced by the host cell in which the virus was grown. This phenomenon was somewhat similar to but not identical with that observed in a study of type III poliovirus, the mouse-adapted Leon strain. In that study(2), the mouse virulence of Leon virus was nullified after 2 passages in MS cells in lactalbumin or bovine plasma hydrolysate medium(4). In the present study virulence of Echo 9 virus was reduced after several passages in the MS cells

CHART 1. Assay of virulence in mice of Echo 9 virus after serial passages in primary or stable monkey kidney tissue culture.



Key for Chart 1

K = Primary monkey kidney tissue culture passage. K_S = Stable monkey kidney tissue culture passage. K_1; K_3; K_{S3}; etc. = First passage in primary monkey kidney tissue culture; third passage in primary monkey kidney tissue culture; third passage in stable monkey kidney tissue culture. M = Mice inoculated intramuscularly. * = Dilution of tissue culture fluid, $\log_{10}$. ** = Log No. of $TCID_{50}$ inoculated into one mouse. § = No. of mice paralyzed or sick over No. inoculated. §§ = No. of mice died over No. inoculated. §§§ = Average survival time (days) of those mice which eventually died.

in Eagle's medium, while virulence was not reduced by passage in MS cells in bovine plasma hydrolysate medium. Kanda and Melnick found that 12 serial passages in MS cells did not affect the monkey neurovirulence of type I poliovirus, (Mahoney)(5). In the present study intracerebral inoculation of 1 ml of undiluted virus (passage level K_{12} and K_{S12}) into each of 4 rhesus monkeys—2 monkeys for virus of each cell line—produced no symptoms or rise of temperature during a period of 21 days nor histopathological lesions in their central nervous system afterwards. Mouse virulence seemed to be independent of monkey virulence.

The present study was aimed at demonstrating that the dependence of viral virulence on the host cell was not confined to type III poliovirus. No attempt was made to purify the Echo 9 virus by the plaque method as was done for the type III poliovirus(2). How the MS cells selected the avirulent or less virulent virus particles was not determined.

The MS cells were found to be harboring pleuropneumonia-like organisms (PPLO) which might, it was thought, influence the virulence of virus. However, in separate studies, IC inoculation of a pure culture of PPLO (10^4 organisms per mouse) did not cause symptoms or death in suckling mice; nor did it influence the activity of a tumor-inducing virus (unpublished). The virus grown in either primary or stable cells was neutralized in TC by anti-Echo 9 rabbit serum. Eggers and Sabin(6) have extended the work of McLean and Melnick(3) and other workers and have reported that the naturally occurring Echo 9 viruses varied in mouse pathogenicity. The virulence problem is complicated and probably a large number of determining factors are involved.

Summary. Serial passages of Echo 9 virus were carried out in 2 sets of monolayer tissue culture in Eagle's medium: (a) in primary monkey kidney (MK) cells (b) in monkey stable, (MS) kidney cells. After several passages, the virus lost most of its virulence for mice in MS cells but not in MK cells.

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