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Sensitivity of Three Cell Lines to Japanese Encephalitis Virus Infectivity Assay

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Abstract: Comparative sensitivity test on three established cell lines for the plaque formation of Japanese encephalitis (JE) virus showed that the highest infectivity was recorded on Vero, followed by BHK21 and then Hep-2 cell lines. The result indicated that these cell lines possess different sensitivity to JE virus in the early stage of infection.

Key words: Japanese encephalitis virus, Infectivity assay, Virus sensitivity

INTRODUCTION

Virus isolation is still the most commonly used method for the diagnosis on viral diseases along with serology, most routinely using cell culture systems with sensitivity and flexibility for virus growth (Mcintosh, 1990). It has frequently been reported that the virus isolation rate for a certain viral disease varied with different cells used in the study (Henchal *et al.*, 1990), indicating the different cell sensitivity. Usually the cell sensitivity to a certain virus has been determined by measuring the virus yield after infection (Igarashi, 1978; Sasaki *et al.*, 1964). This method, however, will not provide an answer to the efficiency of the originally inoculated virus to establish infection in those cells. In our study on infectivity assay of JE virus, we found that the number of plaques formed on three established cell lines was different even with the same amount of virus inoculum. The result indicated the different sensitivity of each cell line to JE virus in the early stage of the infection.

MATERIALS AND METHODS

Viruses: JE virus Nakayama strain, JaOArS982 and JaNAr6584 strains used in the study have been kept in our Department. Seed viruses were prepared in *Aedes albopictus* clone C6/36 cell lines at 28°C (Igarashi, 1978).

Cells: Vero, BHK21 and Hep-2 cell lines were used for plaque assay and focus assay. These cell lines and C6/36 cell line were kept in our Department using cell growth medium of 9% heat-inactivated fetal calf serum in Eagle's medium in Earle's saline sup-

plemented with 0.2 mM each of nonessential amino acids. Three mammalian cell lines were grown at 37°C while C6/36 cells at 28°C, respectively.

Plaque assay: Standard plaque assay method for JE virus in our Department using BHK21 cell line on 24 well semimicroplate with methyl cellulose overlay medium (Shameem, 1989) was applied to Vero and Hep-2 cell lines. The seed virus was diluted in virus diluent (0.2% gelatin in phosphate buffered saline, pH 7.4) and inoculated to cell monolayers (100 μ l/well) from which the growth medium had been removed. After 2 hr of adsorption, the cells were covered by methyl cellulose overlay (1.25% in cell growth medium from which serum concentration was reduced to 1%). The plaques were visualized by staining methanol-fixed cells after 5 days incubation at 37°C in 5% CO₂-atmosphere.

RESULTS

Fig. 1 shows comparative plaque numbers observed on Vero, BHK21 and Hep-2 cell lines, for three JE virus strains, namely Nakayama, JaOArS982 and JaNAr6584, at the same virus dilution. For all three JE virus strains tested, the number of plaques was highest on Vero cell, followed by BHK21 cell and the lowest number on Hep-2 cell.

In the above experiment, the seed viruses were prepared in C6/36 cell line, possessing the envelope possibly containing the host cell membrane components. In order to exclude the possibility that the observed difference in the number of plaques in Fig. 1 was due to the C6/36 cell host factor, similar comparative plaque formation test was performed using JE virus Nakayama strain grown on Vero, BHK, and Hep-2 cells for 2 days at 37°C. The newly

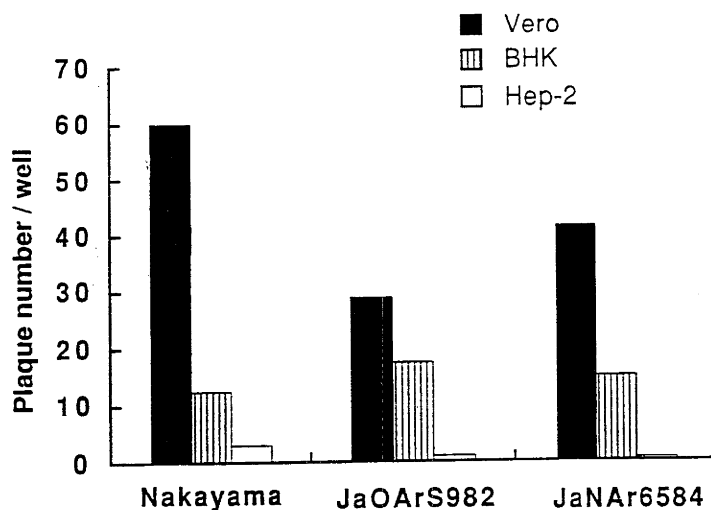


Fig. 1. Comparative sensitivity of JE virus plaque formation on Vero, BHK21 and Hep-2 cell lines.

Three established cell lines, Vero, BHK21, and Hep-2, were inoculated with JE virus Nakayama, JaOArS982 and JaNAr6584 strains prepared in C6/36 cells and were diluted to 10⁻⁵ to form plaques as described in the text.

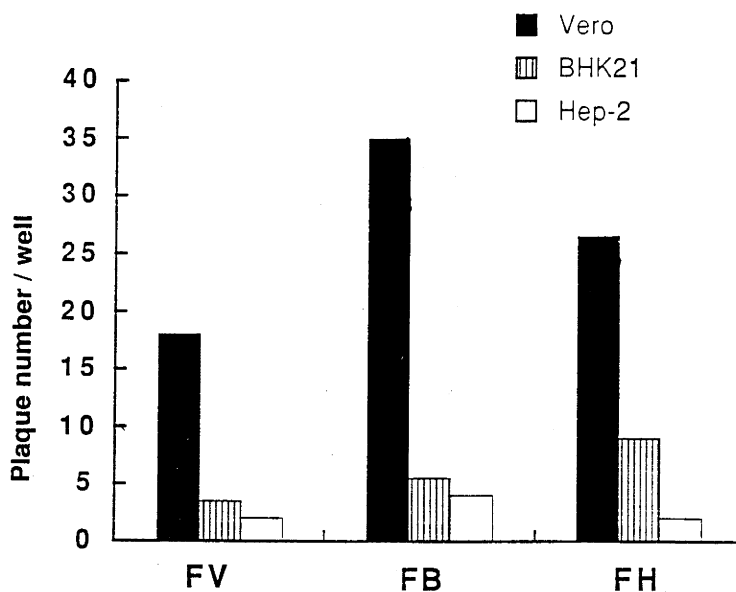


Fig. 2. Comparative sensitivity of JE virus plaque formation on Vero, BHK21 and Hep-2 cell lines, using the Nakayama strain grown in each respective cell line. The seed of the Nakayama strain of JE virus was prepared in Vero (FV), BHK21 (FB) and Hep-2 (FH) cell lines, and then inoculated in each cell line to form plaques as described in the text.

prepared JE virus grown in three different cell lines was inoculated to each cell line to form plaques. The result shown in Fig. 2, demonstrated that the plaque number was highest on Vero cell, followed by BHK21 cell, and the lowest on Hep-2 cell line, regardless the cell line in which the seed virus was prepared.

DISCUSSIONS

It is well-known that under controlled conditions, a single plaque represents one infectious virus particle in the virus preparation originally inoculated to the host cells. Therefore, the plaque numbers formed on a certain cell line can reflect the sensitivity of each cell line to the virus in the early stage of infection. Compared to the conventional cell sensitivity determination by assaying the virus yield, this method could reveal how the originally inoculated infectious virus initiated infection in the early stage of infection. This method could help to explain the different virus isolation rates by using different cell lines in clinical virology. At least one of the reasons may be that for the given amount of virus in the test sample, the possibility to successfully establish infection on sensitive cells is higher than on less sensitive cells in the early stage of infection. Although our result was provided by limited information on JE virus plaque formation, however, the same principle can be applied to other virus-host cell systems including non-plaque forming cells.

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