Plaque Formation of Herpes Virus Hominis Type 2 and Rubella Virus in Variants Isolated From the Colonies of BHK21/WI-2 Cells Formed in Soft Agar

Brief Report

By


1 The 1st Department of Oral and Maxillofacial Surgery, Osaka University, Dental School, Osaka, Japan
2 Osaka Prefectural Institute of Public Health, Osaka, Japan
3 Toyama Institute of Health, Toyama, Japan

With 1 Figure
Accepted November 22, 1976

Summary

Variants isolated from the colonies of BHK21/WI-2 cells formed in soft agar medium supported extremely clear plaque formation of herpes virus hominis type 2 and rubella virus in agar medium as compared in the parental cells.

Some animal viruses released from their infected cells carry components of the host cell membrane as their viral outer coats which are acquired during the processes of viral release. This finding indicates that the functions or nature of the cell membrane should reflect a degree of viral replication. The studies presented in this paper are designed to investigate several important aspects on the function of host cell surface necessary to express the plaque forming ability of the viruses.

For the above reasons, the two representatives of enveloped viruses, herpes virus hominis type 2 and rubella virus, and their common propagating host BHK 21/WI-2 cells, have been employed in these studies. Herpes virus hominis type 2 can be propagated on BHK21/WI-2 cell culture of the established cell line derived from syrian hamster. However, it does not form plaques in agar medium: it induces cytopathic effect (nonspecific rounding cells) but forms no plaques on the BHK21/WI-2 cells. Rubella virus, however, replicates in BHK21/WI-2 cell culture, leading to plaque formation although sensitivity of these cells to rubella virus by plaque formation exhibits wide variation at various cell passage levels.
On the other hand, the cell population in established cell lines including BHK21/WI-2 cells is generally composed of the mixture of untransformed and transformed cells although untransformed rodent cell lines such as BHK21 cells tend to become aneuploid and tumorigenic after serial passage in culture (3). Since transformed cells alone have a colony forming ability in semi-solid agar (1, 4, 6), the cells derived from colonies of BHK21/WI-2 cells formed in soft agar were isolated as selective variants and the plaque forming abilities of these viruses in their cell cultures were investigated.

BHK21/WI-2 cells (8) were cultured at 37°C in a humidified atmosphere composed of 5 per cent CO₂ in air in Eagle minimum essential medium (MEM), supplemented with 2 mM L-glutamine, 10 per cent calf serum and 10 per cent tryptose phosphate broth (Difco Laboratories, Detroit, Michigan, U.S.A.) as growth medium.

Rubella virus strain M-33 (5) and herpes virus hominis type 2 UW-268 strain (9) were grown on BHK21/WI-2 cell cultures and the infected culture media were used as seed viruses after clarification by low speed centrifugation.

The variants derived from the colonies of BHK21/WI-2 cells formed in semi-solid agar were prepared as follows: a total of 500 BHK21/WI-2 cells were mixed with 1 ml of agar medium containing 0.3 per cent Special Agar Noble (Difco Laboratories, Detroit, Michigan, U.S.A.) in growth medium and the mixture was plated onto a basal layer which had been made by pouring 2 ml of 0.6 per cent agar medium into 30 mm Petri dishes. The colonies formed after about 30 day incubation at 37°C in a 5 per cent CO₂ incubator, were isolated with Pasteur’s pipettes and the cells were cultured in 3 ml of Eagle’s growth medium in 30 mm Petri dishes until the formation of confluent cell monolayers. The growth medium was changed twice a week during that period. Out of ten variants isolated, four variants which showed stable growth were used in this study. They were designated as Bh, Bi, Bj and BSR cells, respectively. These variant clones having a rounded type in shape showed an increased growth rate, as compared with the parental cell line. They were stored at -70°C at various cell passage levels until use.

Rubella virus and herpes virus hominis type 2 were assayed by the plaque forming ability in these variant cell monolayers. These cells were plated onto 50 mm Petri dishes at a density of about 10⁶ cells per dish. After two day cultivation at 37°C, the confluent cell monolayers formed were infected with 0.1 ml of an appropriate dilution of seed viruses. At the completion of one hour adsorption, the cell monolayers were washed once with MEM for removal of inoculum and then overlaid with 1 per cent agar medium. The plaques formed after further incubation at 37°C for about 6 days, were counted by staining with 300 X neutral red solution in Dulbecco’s phosphate buffered saline (2).

As shown in Table 1, a greatly increased sensitivity of these variants to plaque formation of rubella virus was found as compared with BHK21/WI-2 cells itself. Particularly, BSR cells carrying the extremely high ability to support the plaque formation of rubella virus (Fig. 1a) exhibited a 25-fold sensitivity to rubella virus infection at the sixth passaged cells in comparison to that of the parental cells.

Since rubella virus formed an increased number of plaques in these colony forming cells, they were tested for the plaque formation of herpes virus hominis type 2 as one of other enveloped viruses than rubella virus. Consequently, this