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GIANT CELL-FORMING VARIANTS OF THE MIYAMA STRAIN OF TYPE 1 HERPES SIMPLEX VIRUS WHICH DIFFER IN FUSION ACTIVITY

SHIRO NII, MASAO YAMADA and FUMIO UNO

Department of Virology, Okayama University Medical School, Shikatacho, Okayama 700, Japan

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During serial passages of a non-giant cell-forming variant (−GCr) of the Miyama strain of type 1 herpes simplex virus, a new giant cell-forming variant named +GC(81) was isolated. CPE induced by this isolate was compared with that by −GCr and also by +GC(LPV), a derivative strain of the +GC variant of the Miyama strain.

Rounding of single cells was observed after infection with −GCr. Remarkable syncytial formation was induced by +GC(LPV), the syncytia containing hundreds of nuclei, while small giant cells were formed by +GC(81). The reason for the appearance of +GC variants that differ in fusion capacity is discussed.

It has been shown that within a few passages in vitro fresh isolates of herpes simplex virus usually do not induce fusion of cultured cells, but that mutants causing syncytial giant cells appear after in vitro cultivation of the isolates for a certain period (Hoggan and Roizman, 1959; Nii and Kamahora, 1961).

The Miyama strain of type 1 herpes simplex virus was originally isolated from a patient with herpes labialis onto an FL cell monolayer. During in vitro passages of this strain, two CPE variants, named +GC and −GCr, were isolated. The former induces giant cell formation while the latter causes rounding of infected cells (Nii and Kamahora, 1961). A third CPE variant named −GCf, which causes fusiform development of infected cells, was also isolated (Nii, 1961). After the first isolation of +GC virus from the original Miyama strain, another +GC virus was isolated after serial passaging of the −GCr virus for about 2 years, suggesting that +GC virus appears from −GCr virus by mutation (Nii and Kamahora, 1962b). In 1970, Niwa isolated two plaque variants from the former +GC strain, named +GC (LPV) and +GC (SPV), respectively; one forms large plaques while the other forms small ones (unpublished results). Syncytial formation induced by +GC (LPV) or by +GC maintained in about 1970 appeared to be somewhat different from that caused by +GC at the early stage of passage after its isolation in 1960. This difference, however, could not be confirmed by comparative experiments using these three kinds of +GC variants, because +GC at low passage level had already been lost.

In 1980, during serial passages of −GCr, a

few foci that consisted of infected cells showing CPE of the +GC type were detected on an infected FL cell monolayer while the rest of the monolayer consisted almost entirely of rounded cells characteristic for infection with −GCr. This suggested that a new giant cell-forming (+GC) variant had emerged by mutation and was contained in a very low ratio in the −GCr virus sample maintained currently in our laboratory. So, we attempted to isolate this new +GC variant using the FL cell culture. This preliminary report describes the procedure for isolation of this variant and also a difference between the cytopathic changes by +GC (LPV) and this isolate.

First, with the aim of isolating the new +GC virus efficiently, we tried to pick up infected cells from foci where giant cells were detected and to inoculate them onto fresh FL cell monolayers. This was done as follows. Samples of 1 ml of the supernatant fluid derived from the above FL cell culture, having a titer of about 10^5 PFU/ml, were inoculated onto three FL cell monolayers in 30 ml Falcon plastic bottles. After 1 h adsorption, the inocula were replaced by maintenance medium consisting of MEM supplemented with 5% calf serum and 0.2% human γ-globulin. Venoglobulin-I, purchased from Green Cross Co. (Osaka), was used as human γ-globulin. MEM solution containing this globulin at 2.5% had a neutralizing antibody titer of 1: 128 by the 50% plaque reduction test using the HF strain of type 1 herpes simplex virus. Maintenance medium containing antibody was used to inactivate cell-free virus in the fluid phase of infected cultures. Use of this medium should reduce the possibility in the subsequent procedure of picking up giant cells harbouring the new +GC virus, of contamination with −GCr virus present in much higher concentration in the fluid phase of infected cultures. Two days after virus inoculation, when CPE became remarkable on the monolayers, the cultures were examined with an inverted microscope and sites showing +GC type CPE were marked on the outside of flasks with magic ink. Cells in the marked portions were scarped off, taken up with a Pasteur pippette and inoculated onto two fresh FL cell monolayers in antibody-free maintenance medium, i.e. MEM supplemented with 5% calf serum. As a result, focal lesions showing CPE of +GC or −GCr type were produced and foci of both types were found on the monolayers in approximately equal numbers.

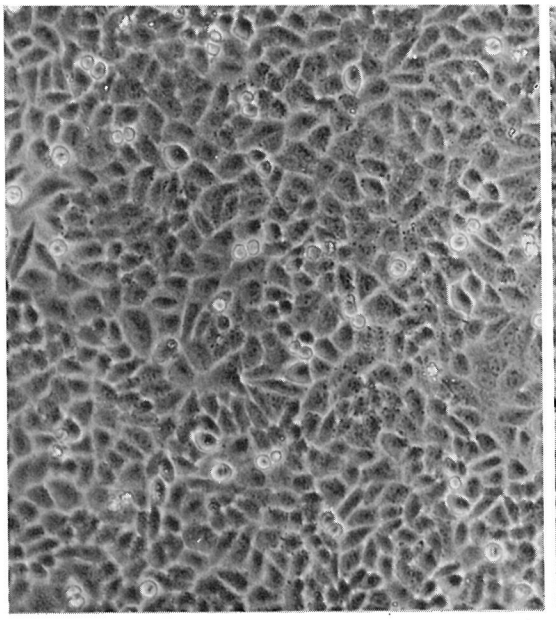
To obtain a viral sample containing the new +GC virus in a much higher ratio, cells infected with this virus were picked up again. When marked CPE appeared in the latter monolayers, the culture fluid was removed. The supernatant fluid obtained by low speed centrifugation was serially diluted tenfold up to 1: 10^6 and 1 ml aliquots of each dilution were inoculated onto FL cell monolayers. After adsorption for 1 h, the inocula were replaced by maintenance medium containing human γ-globulin and the infected monolayers were incubated for 3 days. Several plaques of the +GC type formed at higher dilutions were selected and infected cells in these plaques were scraped off, taken up with a Pasteur pippette and inoculated onto FL cell monolayers in antibody-free maintenance medium. The CPE foci that appeared on

FIGURE 1. 2. 3. 4. FL cells non-infected and infected with −GCr and two +GC variants of the Miyama strain of type 1 herpes simplex virus.

Parallel FL cell monolayer cultures were prepared. Pairs of cultures were infected with −GCr, +GC(LPV) or +GC(81) at an MOI of about 1. Non-infected cultures served as controls. After 2 days, when marked CPE appeared in infected cultures, control and infected FL cells were photographed under a phase contrast microscope. Figure 1 shows control FL cells. Normal looking flattened cells are seen. Figure 2 shows part of an FL cell monolayer infected with −GCr, where many single rounded cells are seen. Remarkable syncytial formation induced by +GC(LPV) is shown in Fig. 3, while many small giant cells formed after infection with +GC(81) are seen in Fig. 4.

these monolayers were shown to be predominantly +GC type.

Using the culture fluid derived from one of the monolayers, the +GC virus was cloned twice by the endpoint dilution method. The new +GC virus strain obtained was named



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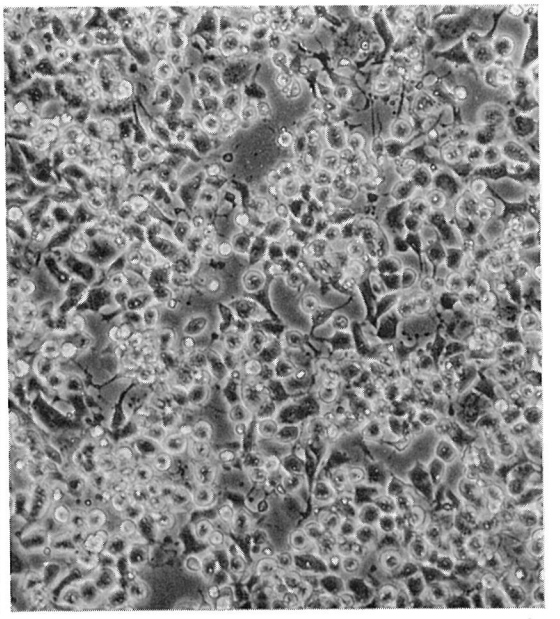

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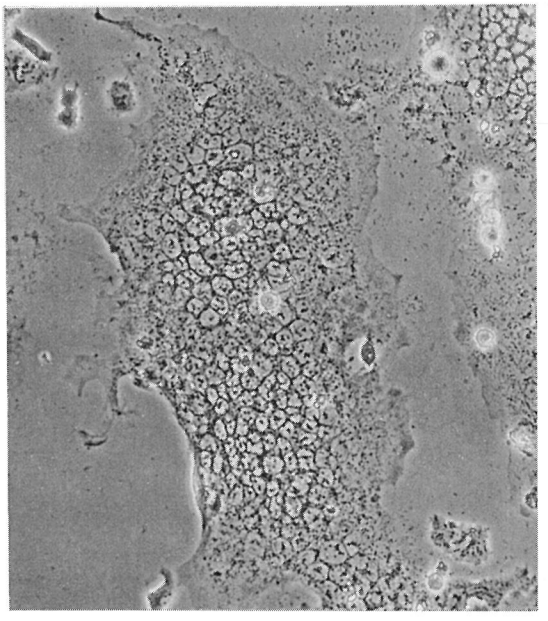

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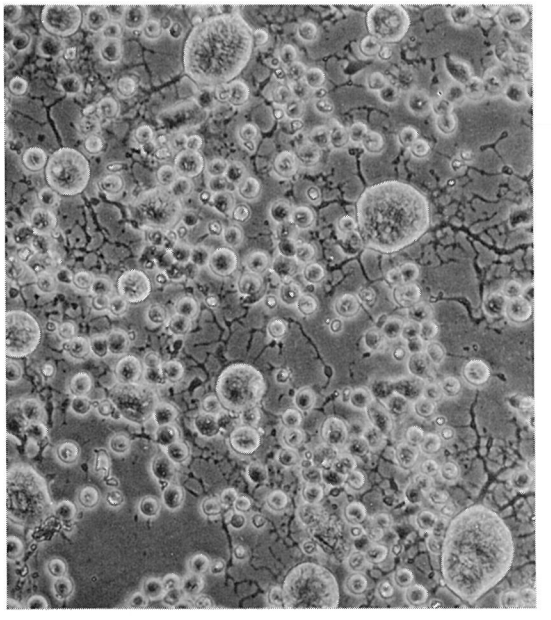

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+GC (81).

The CPE induced by +GC (81) was compared with those by +GC (LPV) and −GCr. FL cells were infected with each variant at an MOI of approximately 1 PFU/cell and when CPE became remarkable the infected and control cultures were observed with an inverted phase contrast microscope and photographed.

Control FL cells are shown in Fig. 1. CPE caused by −GCr in these cells is shown in Fig. 2. Most cells look round. In contrast to this single cell type CPE, +GC (LPV) and +GC (81) both induced cell fusion. The remarkable syncytial formation caused by +GC (LPV) is shown in Fig. 3. A number of nuclei are seen to be agglutinated in a syncytium. In contrast, in FL cells infected with +GC (81) many small round giant cells are seen coexisting with many single rounded cells (Fig. 4). This CPE by +GC (81) was similar to that induced by +GC isolated in 1960, as shown in Fig. 1 of the paper of Nii and Kamahora (1961). This indicates that +GC virus at a low in vitro passage level has lower fusion activity than that after numerous passages. The plaques produced by +GC at an early passage level in vitro were so small that it was necessary to use a microscope to count them exactly (Nii and Kamahora, 1962a), while those formed by +GC virus after numerous passages, such as +GC (LPV) or +GC maintained from about 1970 on, were large enough to be counted by naked eye.

Spread of herpes simplex virus on monolayer cells is greatly influenced by the interaction between infected and non-infected cells. On infection of FL cells with +GC virus at low MOI, fusion occurs between infected and non-infected cells. Accordingly, +GC virus which shows higher fusion activity after infection is thought to cause more efficient transmission of infectious viral particles or macromolecules from infected to non-infected cells through fused portions of cells. This spreading of viral infection through the cytoplasm of fused cells explains why larger plaques are formed by +GC virus with higher fusion activity. During numerous in vitro passages of +GC virus, various variants that differ in fusion activity appear by mutation. Viruses with higher fusion activity spread more rapidly on monolayers and gradually become predominant in the +GC virus population. This idea is substantiated by the fact that +GC at a low passage level after isolation in 1960 and +GC (81) isolated in 1981 produced small syncytial giant cells and formed smaller plaques, while +GC (LPV) isolated after numerous in vitro passages for 10 years induced huge syncytial cells and formed larger plaques.

Thus, in general, +GC with higher fusion activity is thought to have a more selective advantage during serial passages regardless of the presence of neutralizing antibody in the culture medium, although the presence of this antibody may enhance this advantage, as shown in the isolation of the macroplaque variant by Hoggan and Roizman (1959). In the present study, human γ-globulin was used to neutralize infectious virus released into the fluid phase of infected cultures and so to facilitate isolation of +GC virus from giant cells without contamination by −GCr virus in the medium. These giant cells that harbour +GC virus were thought to transmit infection, when inoculated onto fresh monolayers nourished with antibody-free maintenance medium, by fusion with non-infected cells or by releasing infectious virus.

The characteristic of +GC (81) that induces small giant cells was shown to be stable on examination after five serial passages in vitro. Except for the emergence of +GC virus in the −GCr virus population, as shown in this report, and that of some plaque variants in the +GC virus population within a long virus passage period of 10 years, CPE variants of the Miyama strain have consistently manifested their respective characteristic CPE. This indicates that their in vitro markers are very stable. This is in sharp contrast with a rapid variable character of one peculiar strain previously isolated by

one of the authors (Nii, 1972). At present we keep three +GC variants of the Miyama strain, which can be differentiated by the extent of their fusion activity, i.e. +GC (LPV), +GC (SPV) and +GC (81).

To elucidate the mechanism of cell fusion by herpes simplex virus, both viral and cellular factors should be investigated. However, there are no reports of attempts to differentiate syncytia-forming (Syn) variants derived from the same original strain by their fusion capacity and without analytical studies on the Syn character of Syn mutants somewhat discrepant experimental results and conclusions may be obtained. The +GC virus strain obtained long ago and currently used in several other laboratories in Japan and USA seems to have similar biological characteristics to +GC (LPV) described here (Shimizu et al., 1978; Dix, McKendall and Baringer, 1983). The pathogenicities to experimental animals of various Syn variants derived from the same strain may also differ. In fact, three Syn variants of the Miyama strain differ from one another in pathogenicity to mice. Details of these differences will be published elsewhere.

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