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INTERFERENCE WITH POLYOMA VIRUS REPLICATION BY HOMOLOGOUS INCOMPLETE VIRUS

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SUMMARY SVT cells are a continuous cell line isolated from a tumor in a hamster induced by simian virus 40 (SV40). Their infection with polyoma virus resulted in the production of two kinds of progeny. Namely, most of the virus particles were noninfectious but a few were infectious. The defective particles interfered with the growth of infectious polyoma virus in A31 cells, a continuous cell line from BALB/C mice. The sensitivity of defective particles to ultraviolet irradiation suggested that their DNA must be functional for interference to be manifested. This interference was observed even when the defective particles were added 6 hr after complete virus infection and it was not due to death of host cells before maturation of infectious virus particles.

INTRODUCTION

It has been reported that defective particles, with similar antigenicity to the parental infectious viruses, are produced by undiluted passages of influenza virus (von Magnas, 1954), fowl-plaque influenza virus (Rott and Scholtissek, 1963), Rift valley fever virus (Mims, 1956), Vesicular stomatitis virus (Huang et al., 1966) and SV40 containing DNA (Uchida et al, 1966). The defective particles are also produced when some kinds of hybrid cells, 10A, derived from a hybrid between mouse and hamster cells are infected with polyoma virus (Basilico, 1970).

SVT cells, derived from a tumor induced in a hamster with SV40, produce large amounts of defective virus with CPE when infected with polyoma virus (Hakura et al., in preparation). Polyoma virus stocks obtained from SVT cells always show a much lower PFU:HA ratio than that of normal stock prepared from mouse cells. This lower PFU:HA ratio seemed to be due to the large quantities of defective particles in the SVT lysate (Takeuchi et al., to be published). During studies on the defective particles, we found that the particles interfered with the replication of complete polyoma virus in mouse cells. There are several reports which described the interference between homologous complete virus and the defective particles (Bellett and Cooper, 1959; Rainbow and Mak, 1970), but little is known about the mechanism of this phenomenon.

This report is on the interference of homologous defective particles with polyoma virus.

MATERIALS AND METHODS

1. *Growth medium*

All cultures were grown in Eagle's minimum essential medium (MEM) supplemented with 5–10% calf serum.

2. *Virus*

SE-polyoma virus (strain 105) was used. For convenience, the virus stock is referred to as M-PV. The M-PV stocks used in this work were mainly prepared using A31 cell cultures. Winocour's method was employed for preparation of stock of high titer, (Winocour, 1963). Polyoma virus stock grown in SVT cells (SH-PV) was prepared as follows. Suspensions of SVT cells were inoculated with M-PV at a multiplicity of infection of 10^2, and incubated at 37 C for 2 hr with occasional agitation. Then the cells were washed 3 times and mixed with antiM-PV serum. After one hour, these infected cells were washed to remove the antiserum and 5×10^6 cells were inoculated into Roux bottles with fresh medium containing 5% calf serum. After incubation for 4 days at 37 C, most of the infected cells showed cytopathic changes and were detached from the glass bottle. The culture medium containing these detached cells was subjected to ultrasonic vibration and used as SH-PV stock.

3. *Cells*

SVT cells: A stable line from a tumor induced in a hamster by SV40 isolated by Dr. K. Toyoshima in this laboratory.

A31 cells: A continuous cell line from BALB/C mice, kindly provided by Dr. P. K. Vogt. The A31 cells used in this study were a subline of the original A31 cells, prepared by Dr. T. Kakunaga (Kakunaga and Miyashita, 1972).

4. *UV-irradiation of virus*

Polyoma virus was suspended in 1.5 ml of MEM and irradiated in a petridish (40 mm diameter) with occasional shaking from an distance of 15 cm using a 30 w Toshiba germicidal lamp.

5. *Hemagglutination (HA) test*

The hemagglutinin (HA) test was performed as described by Eddy (Eddy et al., 1958). Before incubation at 4 C solutions used for dilution were usually warmed to 37 C to obtain maximum elution of hemagglutinin from inhibitors in the culture fluid.

6. *Interference test*

Prescription bottles containing confluent cell layers were inoculated with SH-PV and M-PV viruses simultaneously. After incubation for 2 hr at 37 C, free viruses were removed by washing the cells three times and then fresh medium was added to the cultures. Then at 24 hr intervals, the viruses replicated in the cultures were titrated by the hemagglutination method or the plaque assay method after disruption of culture cells by sonication.

RESULTS

1. *Infection of the SH-PV lysate*

SH-PV stock (1,280 HA units) was inoculated onto monolayer cultures of A31 cells. This stock contained 2.7×10^5 PFU of infectious virus.

After removal of uninfected virus, production of virus particles was followed by the HA test at 24 hr intervals. As shown in Fig. 1, no replication of infectious virus in the stock was observed even though the stock contained 2.7×10^5 PFU of infecious virus.

On the other hand, when normal polyoma virus obtained from mouse cells was inoculated into A31 cells with the same plaque forming units, virus multiplication was seen. Multiplication was first detectable after 2 days,

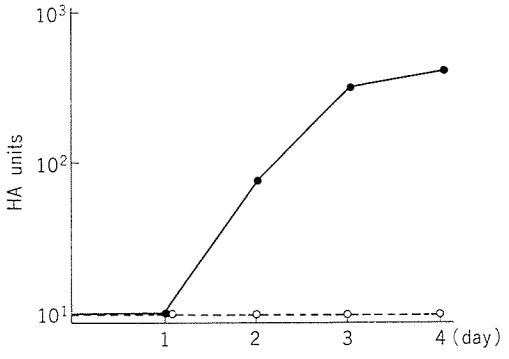

FIGURE 1. *Single-cycle growth of polyoma virus in A31 cells infected with SH-PV or M-PV stock.* ●——● *M-PV (m.o.i. 0.76).* ○-----○ *SH-PV (m.o.i.* 0.76)

and reached a maximum after 3 days.

These results suggest that some factors in the SH-PV stock interfere with replication of infectious virus.

2. *Interference with replication of M-PV by SH-PV stock*

The interference of SH-PV stock with replication of M-PV in A31 cells was tested.

Undiluted SH-PV (1,280 HA units) and tenfold dilutions of this were inoculated onto monolayers of A31 cells together with M-PV virus (multiplicity=500). After adsorption for 2 hr at 37 C, free viruses were removed by washing the monolayers three times and then incubated at 37 C. Virus replication in these cultures was measured every day by the hemagglutination technique. Table 1 shows

TABLE 1. *Effect of SH-PV stock on the replication of M-PV in A31 cells*

SH-PV (HA units)	M-PV	HA units after virus infection (Days)		
		1	2	4
—	+[a]	<10	240	10,240
1,900	−[b]	<10	20	80
1,900	+	<10	40	320
190	+	<10	640	1,920
19	+	<10	320	10,240
1.9	+	<10	320	7,680
0.19	+	<10	320	5,120

Input multiplicity of M-PV: 500 (PFU/cell)
[a] Infected with M-PV only
[b] Infected with SH-PV only

the effects of undiluted and diluted SH-PV stock on the growth of M-PV. The final HA titers of the A31 cultures infected simultaneously with M-PV and undiluted SH-PV were only one eighty eighth of that of the control, infected with M-PV alone. On dilution of SH-PV stock, its interference with M-PV replication decreased and no interference was observed on 100-fold or more dilution.

Thus SH-PV stock interferes with complete virus present in the stock itself and also with replication of other complete virus (M-PV).

3. *Interfering factor in SH-PV stock*

The nature of the factors in SH-PV stock responsible for interference with growth of infectious virus was studied. To see whether the factor was some viral particles in the stock or another substance, such as interferon, the interference of partially purified virus was tested.

Cell debris in SH-PV stock was removed by low speed centrifugation (4,000×g, 30 min) and the resulting supernatant was recentrifuged at 30,000 rev/min for 120 minutes in a Hitachi Rp 30 rotor. Almost all the virus particles were found in the pellet fraction. Interference by the supernatant and pellet fractions were tested as described in the materials and methods. The yields of polyoma virus 4 days after infection, shown in table 2, indicate that only the pellet fraction caused interference. Thus it is unlikely that the interference is caused by a substance such as interferon.

TABLE 2. *Interference of pellet and supernatant fractions of SH-PV stock*

Virus	Final yield of polyoma virus (HA units)
M-PV	1,280
SH-PV (Whole)	<10
SH-PV (Pellet)	<10
M-PV+SH-PV (Whole)	60
M-PV+SH-PV (Pellet)	40
M-PV+SH-PV (Sup.)	1,280

Input multiplicity of M-PV, 109. 1,280 HA units of SH-PV (whole) and SH-PV (pellet) were used for infection.

4. *Effect of UV-irradiation on interference by SH-PV virus*

Two experiments were performed to test the effect of UV-irradiation of interference by SH-PV virus.

In the first experiment, both SH-PV and M-PV stock were irradiated by UV-light.

The survival curves of M-PV and of infectious virus in the SH-PV stock after different periods of UV-irradiation, shown in Fig. 2, indicate that the sensitivities of the two viruses to UV-light were the same.

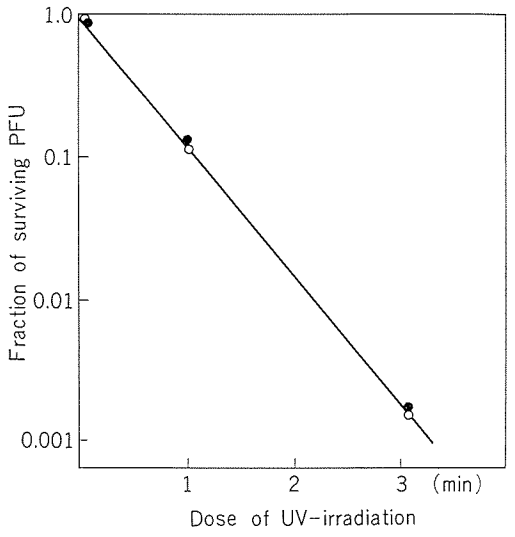

FIGURE 2. *Effect of period of UV-irradiation on replication of infectious virus in M-PV and SH-PV stocks.* ○, *SH-PV.* ●, *M-PV.*

In the second experiment, the effect of UV-light on the interference of SH-PV stock with replication of M-PV was examined. A31 cells were infected with SH-PV viruses which had been irradiated with various doses of UV-light, together with a constant number of unirradiated M-PV virus particles and unirradiated SH-PV virus was used as a control. After adsorption for 2 hr at 37 C, the infected cells were washed three times with MEM and incubated at 37 C and their virus titer was tested every day by HA titration. The results are shown in Fig. 3.

The interference of SH-PV virus with M-PV replication decreased with increase in the period of UV-irradiation, and interference was completely lost after 3 minutes irradiation under the conditions used in this experiment.

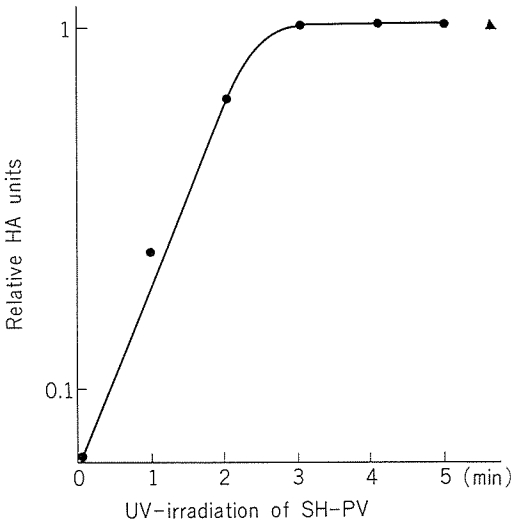

FIGURE 3. *Effect of the period of UV-irradiation of SH-PV on its interference with replication of M-PV. Monolayers of A31 cells were infected with SH-PV stock which had been irradiated for various periods with UV-light and a constant titer of M-PV. After 2 hr incubation at 37 C infected cultures were washed three times with MEM to remove free virus. Then the cultures were reincubated at the same temperature, and their HA titers were measured daily. Their titers 4 days after infection* (●) *were compared with those of cultures infected with M-PV only,* (▲).

5. *Time of interference*

Next studies were made on the stage of the replicative cycle of M-PV which was sensitive to interference by SH-PV virus.

Monolayer cultures of A31 cells were infected with M-PV at a multiplicity of 100. After adsorption for 2 hr, the infected cultures were washed and incubated at 37 C. At intervals, 3 infected cultures were superinfected with SH-PV stock. These cultures were then washed after incubation for two hours at 37 C and reincubated.

In the control uninfected medium was used instead of SH-PV stock. The yield of virus in infected cultures was measured every day by HA titration. The yields of virus after 4 days are shown in Fig. 4. As mentioned previously, after simultaneous

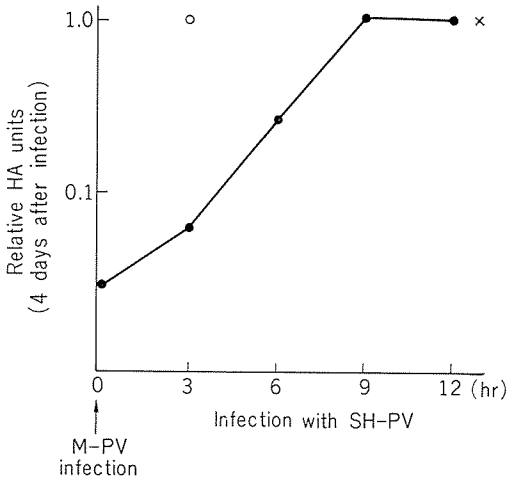

FIGURE 4. *Effect of superinfection with SH-PV on replication of M-PV. Monolayers of A31 cells were infected with M-PV (m.o.i. 100). Three cultures were infected with SH-PV at 0 time. Two hr later unadsorbed free virus was washed out. At 3 hr intervals these cultures were infected with SH-PV stock (HA 1,280). Released virus was measured daily by HA titration. The titer of released virus reached a maximum after 4 days and the culture fluid was harvested. Cell sheets were harvested, mixed with these fluid, and sonicated and then their HA titer was measured, (●). The final titers of control cultures infected with M-PV only (×) and those of cultures superinfected 3 hr later with SH-PV which had been treated with anti SH-PV serum (○) are also indicated.*

infection with M-PV and SH-PV at O time, the virus yield was greatly reduced. Results show that SH-PV caused interference until 9 hr after M-PV infection.

The effect of anti-serum on interference by SH-PV stock was also examined.

SH-PV stock was mixed with anti SH-PV serum and used to superinfect cell cultures which had been infected with M-PV 3 hr previously. Results are also shown in Fig. 4. The interference of SH-PV with replication of M-PV was completely neutralized by anti SH-PV serum. Anti M-PV serum caused similar neutralization of interference. These results suggest that the interference of SH-PV stock with M-PV replication is caused by polyoma virus particles in the SH-PV stock.

6. *Absence of lethal effect of SH-PV stock on host cells*

Studies were made to see whether the reduction in the yield of M-PV caused by SH-PV was due to a lethal effect of the latter on the host cells before maturation of infectious virus. Suspensions of A31 cells were infected with equal HA units of M-PV and SH-PV stocks, and as a control, medium alone was added in place of the viruses. After incubation for 2 hr at 37 C, the infected cells were washed with medium and appropriate dilutions were plated on plastic dishes with growth medium containing anti M-PV serum. After incubation for 10 days at 37 C, the numbers of surviving colonies were counted. As shown in Table 3, the number of surviving colonies of A31 cells after infection with M-PV was one-tenth of that of control cells.

TABLE 3. *Effects of SH-PV and M-PV viruses on colony formation by A31 cells*

Cells	Virus	HA units	PFU	No. of colony formers
A31 (5.5×10^5)	—	—	—	2.4×10^5
	SH-PV	1,280	2.1×10^5	2.0×10^5
	M-PV	1,280	1.2×10^8	2.8×10^4

Under these experimental conditions, there was no significant difference between the colony forming abilities of control cells and cells infected with SH-PV stock.

These results show that the interference by SH-PV stock with M-PV replication in A31 cells was not due to a lethal effect of the SH-PV stock on the host cells before multiplication of coinfected complete virus.

DISCUSSION

SVT cells are a continuous cell line isolated from a tumor in a hamster induced by SV40. They are very susceptible to polyoma virus. Most virus particles obtained from SVT

cells infected with polyoma virus are defective particles, but a few are complete virus particles. The presence of the complete virus in SH-PV stock was deduced from the presence of plaque formers in the stock and the complete replicating ability of virus isolated from these plaques. However, the defective particles could not be separated from the complete virus particles because they were similar to the latter in density and size.

The interference with replication of complete virus was concluded to be due to defective particles in the SH-PV stock for two reasons. First, the ability of SH-PV to interfere with the replication of the complete virus could be seen in the partially purified virus fraction (Table 2). Only the fraction containing defective particles caused interference, while empty particles obtained after density gradient centrifugation did not (to be published). Second, the interference by virus particles of SH-PV stock was completely inhibited by treatment with anti-polyoma serum.

Experiments on the time of interference clearly showed that the replication of complete virus was affected by SH-PV stock for more than 6 hr after infection. This suggests that the period which is sensitive to interference by SH-PV virus is not the adsorption stage, virus penetration or uncoating of complete virus, but the stage after these.

The mechanism of interference is not yet known. The interference with replication of complete virus by defective virus may be caused by some information transcribed from DNA of the defective particles for the following reasons. (1) Most defective virus particles in SH-PV stock are adsorbed to A31 cells normally and can form their own T-antigen (to be published elsewhere), and (2) The interference of this defective virus is a viral function, since it can be neutralized by antibody and inactivated by UV-irradiation (Fig. 3). From these findings and the lack of a lethal effect of SH-PV stock on host cells, it is concluded that defective particles interfere with some system regulating the growth cycle of normal virus.

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