Cell-Mediated Complementation of Human Adenoviruses by Simian Papovavirus[1] (34351)

M. A. JERKOFSKY[2] AND F. RAPP[3]

Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas 77025

Previous studies [reviewed in (1)] have characterized the complementation of human adenoviruses by simian papovavirus SV40 in simian cells; in the presence of the superinfecting SV40 virus, the human adenovirus abortive replicative cycle in the simian cells is converted into a productive one. The factor or factors supplied by the unrelated SV40 virus that permits the human virus to complete its replicative cycle is still unknown. It has, however, been shown that the human adenovirus induces the early, adenovirus-specific tumor or T antigen (2, 3), stimulates thymidine kinase activity (4, 5), replicates its DNA (6, 7), and forms messenger RNA (8) in the nonpermissive simian cells.

The previous studies had been carried out in primary African green monkey kidney (GMK) cells. We have now studied the complementation of these two viruses in stable cell lines originally derived from green monkey kidney cells. The results suggest that the host cell may play a role in the interaction of these two viruses.

Materials and Methods. Viruses. The human adenovirus type 7 (H) was obtained from Dr. M. Benyesh-Melnick who had isolated it from clinical specimens; it had been passed three times in human embryonic kidney (HEK) cells in our laboratory. The SV40 virus was the Baylor reference strain. It had been passed seven times in GMK cells, purified by centrifugation in a gradient of cesium chloride, plaque-purified twice in CV-1 cells, and then passed three times in CV-1 cells. The PARA-adenovirus 7 virus, originally described in Ref. (9–11), was passed two times in GMK cells.

Cells. Primary GMK cells were grown in Melnick's lactalbumin hydrolysate medium (M-H) with 2% calf serum and 0.08–0.23% $NAHCO_3$. All media contained 100 units of penicillin and 100 μg of streptomycin/ml.

Three stable lines of GMK cells were used in these studies. The BSC-1 cells were obtained from Dr. R. Dulbecco, Salk Institute; Vero cells were obtained from Dr. W. E. Rawls. Both of these cell lines were grown in Eagle's minimal medium with 10% fetal calf serum, 10% tryptose phosphate broth, and 0.08–0.23% $NaHCO_3$. The CV-1 cells, obtained from Dr. S. Kit, were grown in the same medium without the tryptose phosphate broth. Primary HEK cells were grown in Eagle's minimal medium with 10% calf serum and 0.08% $NaHCO_3$.

Virus assay. Human adenovirus 7 yields were determined by plaque assay in HEK cells growing in 35 × 10-mm plastic petri dishes. Tenfold dilutions of the virus suspension were made in tris(hydroxymethyl) aminomethane (Tris)-buffered saline, pH 7.4. The virus was added to replicate plates in 0.1 ml amounts and allowed to adsorb to the cell sheet for 1 hr at 37° with occasional manual rotation of the plates. Then, 1.5 ml of an overlay containing 10% fetal calf serum 0.23% $NaHCO_3$, and 1% agar in Eagle's medium was added. One week later, a second

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[2] Predoctoral fellowship No. 1-F1-GM35,287-01 from the National Institutes of General Medical Sciences, National Institutes of Health. Present address: Department of Microbiology, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033.

[3] American Cancer Society Professor of Virology; present address: Department of Microbiology, The Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033.

overlay containing a 1/20,000 dilution of neutral red was added. Plaques were counted on the eighth and tenth days after inoculation.

The yields of SV40 were determined by plaque assay in BSC-1 cells. The cells were grown in 60 × 15-mm plastic petri dishes and 0.3 ml of Tris-buffered saline was added to each plate as carrier fluid. The plaque assay was the same as that above, except that the overlay was added in 5-ml amounts.

Since the PARA-adenovirus 7 population contained two types of particles, each component was titrated independently. The adenovirus 7 component was titrated in HEK cells as described above; the PARA component was titrated in GMK cells that had been saturated with a lawn of a nonreplicating human adenovirus (1).

Growth analysis studies. Cells were grown to form a monolayer in 1-oz prescription bottles. The virus was added in 0.1-ml amounts and allowed to adsorb to the cells for at least 1 hr at 37° with occasional manual rotation. Unadsorbed virus was removed by washing the cell sheet with Tris-buffered saline. Then 5 ml of medium containing 2% fetal calf serum and 0.08% $NaHCO_3$ in Eagle's medium were added and the cultures were incubated at 37°. At each designated time, two culture bottles were removed and the cells were disrupted by two alternate cycles of quick-freezing in an alcohol–dry ice bath followed by thawing at room temperature or at 37°. The cell debris was removed by low speed centrifugation, the supernatant fluids were pooled, and aliquots were stored at —70° until titrated for virus content.

Results. Replication of SV40 in primary and stable lines of GMK cells. The first series of experiments compared the replication of SV40 in primary and stable lines of GMK cells. The stable cell lines included CV-1, BSC-1, and Vero cells. Each cell line was grown to form a monolayer in 1-oz prescription bottles. Cell counts showed that between 5 and 8 × 10^5 cells were present in each bottle. Since the same virus stock was used as the inoculum in comparative experiments, the multiplicity of infection (which ranged from 3 to 5) was similar for all of the cell systems. All comparative experiments were performed at the same time, to insure uniform conditions.

The replication of SV40 virus in each cell system is presented in Fig. 1. In the primary GMK cells, the SV40 had a latent period of 24–32 hr. There was a sharp increase in titer to 48 hr and a somewhat slower increase to 96 hr. The replicative pattern in CV-1 and Vero cells was almost identical to that in the primary cells; the final virus yields were also very similar. In BSC-1 cells, although there was an initial increase in titer between 32 and 40 hr with a slower increase to 96 hr, the titers were about 20-fold lower than those in the other cell systems. This was confirmed in numerous experiments. Both the SV40-specific tumor and viral capsid antigens were readily detected in all of the cell systems by the immunofluorescence technic (11).

Replication of adenovirus 7 in primary and stable lines of GMK cells. Since the simian virus was capable of replication in all of the

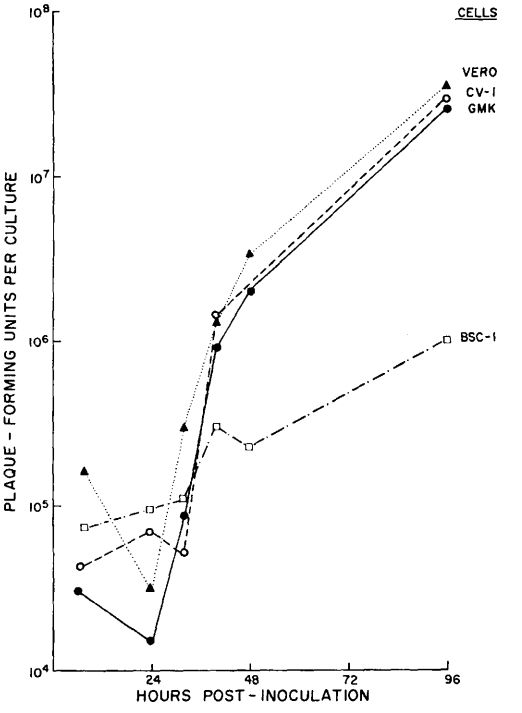

FIG. 1. Replication of SV40 virus in primary GMK cells and in the stable cell lines Vero, CV-1, and BSC-1.

cell systems, the replication of the human adenovirus 7 in each cell system was then determined. The results are presented in Fig. 2. In the primary GMK cells, there was a slow increase in virus titer to 96 hr, with the maximum increase occurring between 48 and 96 hr. The total amount of virus recovered from the cultures was slightly lower than the amount present in inoculum. In Vero cells, the replicative cycle was very similar to that in the primary cells; the major increase in virus titer occurred between 48 and 96 hr and, although the final titer was somewhat higher than that in the primary cells, it did not exceed the amount present in the inoculum. This characteristic of the abortive cycle of adenovirus 7 in simian cells had been noted previously (12). The amount of virus recovered from CV-1 and BSC-1 cell cultures did not exceed the amount of uneclipsed virus present at 6 hr. Thus, the adenovirus 7 was not capable of independent replication beyond input inoculum in any of the stable cell lines.

Complementation of adenovirus 7 by SV40 in primary and stable lines of GMK cells. Since the replicative cycles of adenovirus 7 and SV40 in the various cell systems had been determined, cell cultures were mixedly

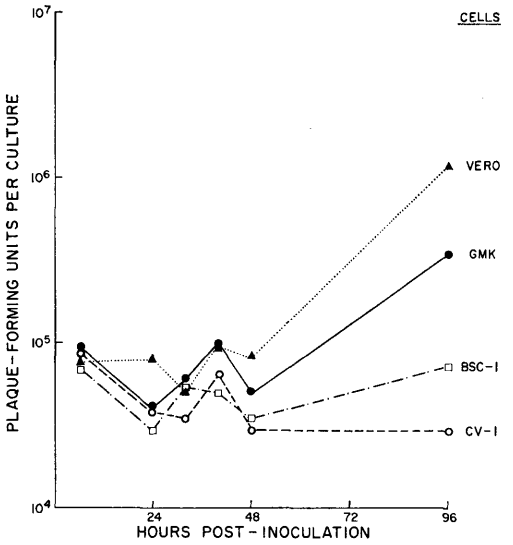

FIG. 2. Replication of human adenovirus 7 in primary GMK cells and in the stable cell lines Vero, CV-1, and BSC-1.

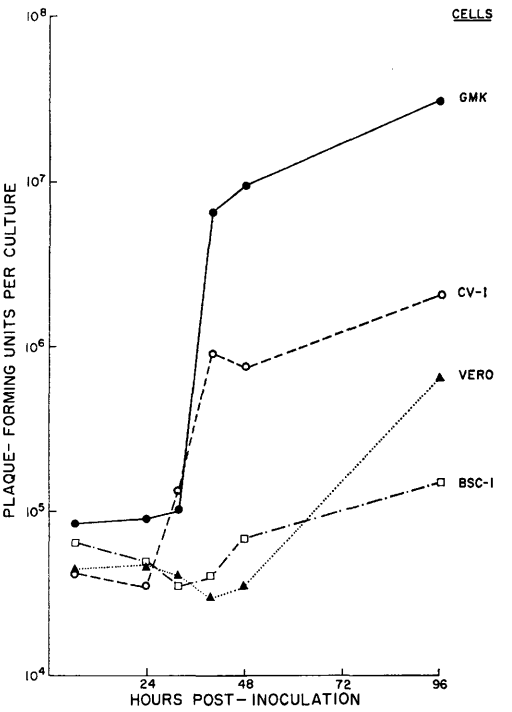

FIG. 3. Replication of human adenovirus 7 in primary GMK and stable cell lines Vero, CV-1, and BSC-1 cells coinfected with SV40 virus.

infected with adenovirus 7 and SV40. The yields of each virus were determined separately. The yields of adenovirus 7 under these conditions of mixed infection are presented in Fig. 3. In the primary GMK cells, the adenovirus 7 had a latent period of 32 hr. There was a sharp increase in titer between 32 and 40 hr and only a small increase after that time. The final yield at 96 hr was 100-fold higher in the presence of SV40 than it was in the absence of SV40. The experiments presented in Figs. 2 and 3 were performed at the same time with the same cell cultures and virus inocula so that direct comparisons of titer could be made. In CV-1 cells, there was an increase in titer between 24 and 40 hr but the titer remained fairly constant after that time. Although the increase in titer in CV-1 cells occurred at the same time as in the primary GMK cells, the titers were about 10-fold lower in the CV-1 cells. However, the titer of adenovirus 7 in CV-1 cells mixedly infected with SV40 were 30-fold higher than those from cell cultures

inoculated with adenovirus 7 alone (see Fig. 2).

In Vero cells, the increase in titer did not occur until after 48 hr and the replicative cycle was almost identical to that shown previously in the absence of SV40. The final titer of adenovirus 7 in cells coinfected with SV40 (6.5×10^5 PFU/culture) showed no increase above the level of virus produced by singly infected cells (1.1×10^6 PFU/culture). In BSC-1 cells, the titer of adenovirus 7 detected at 96 hr in mixedly infected cultures was only slightly higher than the titer at 6 hr. However, in an occasional experiment, a slight stimulation of adenovirus 7 yields could be detected in BSC-1 cells coinfected with SV40. However, the increase in titer was always smaller than that detected in primary GMK cells.

The yields of SV40 in these experiments were also determined and are presented in Fig. 4. The replicative cycle of SV40 in the presence of adenovirus 7 was very similar to that of SV40 alone. The replication of SV40

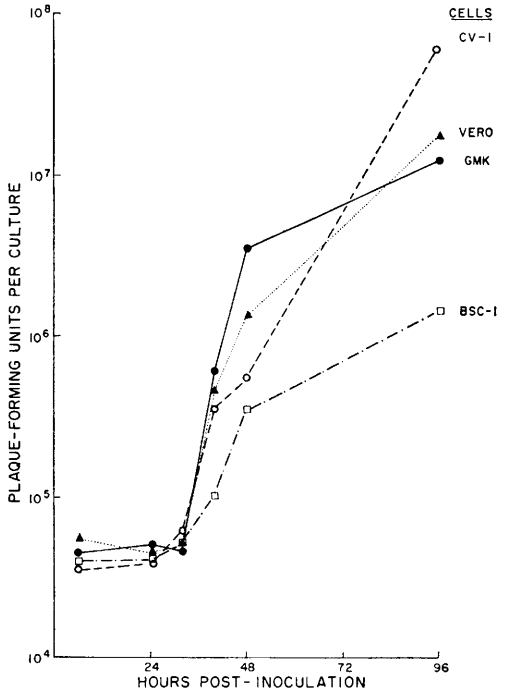

FIG. 4. Replication of SV40 virus in primary GMK and stable cell lines Vero, CV-1, and BSC-1 cells coinfected with human adenovirus 7.

TABLE I. Replication of PARA-Adenovirus 7 in Primary GMK Cells and in the Stable Cell Lines CV-1, Vero, and BSC-1.

Cell system	Postinoculation (hr)	(PFU/culture) Adenovirus yields[a]	(PFU/culture) PARA yields[b]
GMK	40	4.0×10^6	6.5×10^5
	96	7.0×10^6	1.2×10^6
CV-1	40	6.0×10^5	6.0×10^3
	96	1.5×10^6	6.0×10^4
Vero	40	2.0×10^4	6.5×10^3
	96	8.0×10^5	7.5×10^4
BSC-1	40	8.0×10^3	6.0×10^3
	96	8.5×10^4	3.0×10^4

[a] Input was 2.5×10^5/culture.
[b] Input was 2.0×10^4/culture.

in GMK, CV-1, and Vero cells was again quite similar but as before, lower titers were detected in BSC-1 cells.

Replication of PARA-adenovirus 7 in the various GMK cell systems. The PARA-adenovirus 7 population has been shown to contain two types of particles, a human adenovirus 7 component and defective SV40 genetic material encased in an adenovirus 7 protein coat (13, 14). Neither component can replicate autonomously in the GMK cells; both particles are required for a productive replicative cycle in the simian cells (15, 16). The pattern of replication of this adenovirus 7 population carrying the defective SV40 genome was determined in each of the cell systems. The results are summarized in Table I. The titers of each component of the PARA-adenovirus 7 population were determined independently.

In primary GMK cells, the titers of the adenovirus 7 component increased rapidly so that by 40 hr, almost maximum titers were detected. Less than a twofold increase in titer occurred between 40 and 96 hr. Similar results were obtained in CV-1 cells; the adenovirus 7 component had already increased in titer by 40 hr and only a twofold increase was detected at 96 hr. However, the adenovirus 7 yields in CV-1 cells were about fivefold lower than the yields from primary GMK

cells. In Vero and BSC-1 cells, no real increase in titer was detected at 40 hr; the increase occurred between 40 and 96 hr. The final titer of the adenovirus 7 component in Vero cells was similar to the amount present in the virus inoculum. The final titer of virus produced in BSC-1 cells was somewhat lower than input although there was a 10-fold increase in titer between 40 and 96 hr.

The replication of the PARA component in the primary GMK cells closely paralleled that of the adenovirus 7 component. Good replication was detected by 40 hr and a twofold increase occurred between 40 and 96 hr. No increase in the yields of the PARA component were detected in any of the stable cell cultures at 40 hr. A 10-fold increase in titer occurred in CV-1 and Vero cells between 40 and 96 hr with a somewhat smaller increase detected in BSC-1 cells. The pattern of replication of PARA-adenovirus 7 in each cell system was similar to that of mixtures of adenovirus 7 and SV40 except that the defective SV40 component was not replicated as efficiently in the stable cell lines as was complete SV40.

Discussion. Comparison of the complementation of human adenovirus 7 by simian papovavirus SV40 in primary and stable lines of GMK cells suggest that the cell system may influence the interaction of these two viruses. Maximum complementation was detected only in the primary GMK cells although consistent complementation at a lower level was detected in CV-1 cells. No increase in adenovirus titer was detected in Vero cells superinfected with SV40. In BSC-1 cells, only an occasional small increase in titer could be detected in the presence of the simian helper virus. The replication of the SV40 virus was identical in primary GMK cells and the stable cell lines CV-1 and Vero. Therefore, the mere replication of SV40 in a cell system is not sufficient to insure effective complementation of the replication of the human adenovirus.

One possible explanation for the observed difference in complementation is that the adenovirus 7 abortive cycle is different in each of the simian cell systems. Sambrook *et al.* (17) reported that a rabbitpox virus mutant has a different cycle of replication in different cell systems. However, the replication of adenovirus 7 in Vero cells appears to more closely follow the pattern in the primary GMK cells and yet no complementation is detected whereas complementation occurs in CV-1 cells although little, if any, adenovirus 7 is produced in abortively infected cells. This possible explanation cannot be excluded until the adenovirus markers are checked in each of the stable cell lines. Such studies are now in progress. Schell *et al.* (18) have already reported that the adenovirus capsid antigen can be detected in Vero cells using complement-fixation tests.

One other possible explanation is that the factor or factors produced by SV40 that permits complementation of adenovirus replication is not essential for replication of SV40, although it is required for complementation to occur. Although it is not likely that a small virus such as SV40 possesses nonessential genetic information, the SV40 could permit the complementation by derepressing a host cell cistron. This host cell function would be present and capable of expression in the primary cells. However, in the evolution of certain stable cell lines from primary cells, such as Vero, cells capable of expressing this function may have been lost. With other cell lines, such as CV-1, these cells may have been retained. At the present time, there is no evidence to select between these possibilities.

The patterns of complementation detected in cells mixedly infected with adenovirus 7 and SV40 were paralleled in cells infected with PARA-adenovirus 7, a hybrid population containing adenovirus 7 and a defective SV40 genome. The use of the simian adenoviruses SA7 and SV15 as helper viruses did not essentially change the observed patterns (personal observations).

Summary. Although SV40 can replicate well in certain stable cell lines of GMK cells, some of the infected cultures do not support the complementation of the human adenovirus 7. Maximum complementation occurred only in the primary cells although consistent complementation at a lower level was detected in CV-1 cells. No complementation oc-

curred in Vero cells and little, if any, was detected in BSC-1 cells. Therefore, the host cell has a role in determining the interaction of these two viruses and conditions other than those necessary for the efficient replication of SV40 must be present for the complementation of the human adenoviruses in simian cells.

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