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# Isolation of a Variant of Human Adenovirus Serotype 2 That Multiplies Efficiently on Monkey Cells

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A variant of adenovirus serotype 2 (Ad2) that overcomes the block to multiplication of the wild-type Ad2 on monkey cells is described. The variant was selected, after nitrous acid mutagenesis, by sequential passage on monkey cells. This variant forms plaques with similar efficiency on human and monkey cells. The kinetics of its growth and its burst size on human and monkey cells are similar. These growth properties are similar to those found for wild-type Ad2 on monkey cells when the block is overcome by coinfection with simian virus 40.

Early events during lytic infection by human adenovirus appear to occur normally in monkey cells (3, 5, 7, 12, 14). The synthesis of late adenovirus proteins in these cells, however, is abnormal (1, 2, 5–8, 10), and the infection is abortive, with approximately a 1,000-fold lower yield of virus than that in a parallel permissive infection of human cells. The block to multiplication can be overcome if certain genetic information from simian virus type 40 (SV40) is present in the adenovirus-infected monkey cells either (i) in the form of the entire SV40 virus, as in coinfection (11), (ii) in the form of an integrated genome as in SV40-transformed cells (7), or (iii) as part of the adenovirus genome as found in certain adenovirus serotype 2 (Ad2)-SV40 hybrids such as Ad2$^+$ND1 (6, 9).

A new variant of Ad2 that is able to multiply efficiently in monkey cells without the aid of SV40 has been selected. The variant was obtained by treating a high-titer stock of Ad2 (0.2 ml of $3 \times 10^{10}$ PFU/ml) with nitrous acid, as described previously (4, 15), and then by neutralizing the reaction mixture with four volumes of 200 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer (pH 8.75) at 0°C after 3 to 8 min at 22°C. A portion of the resulting mutagenized stock was titrated on HeLa cells to determine the survival level (Fig. 1). A second portion was applied to a monolayer of $CV_1$ (an established line of African green monkey kidney [AGMK] cells) that had just reached confluency. After 1 h at 37°C, the monolayer was washed with phosphate-buffered saline (PBS), and 5 ml of fresh medium (Dulbecco modification of Eagle minimal essential medium supplemented with 10% fetal bovine serum, 100 μg of streptomycin per ml, 100 μg of penicillin per ml, and 20 μg of mycostatin per ml) was added. After 3 days, the cells were harvested by suspension in 0.5 ml of PBS and lysed by freezing, thawing, and sonic treatment, and applied to a fresh monolayer of $CV_1$. After 1 h at 37°C, the monolayer was washed with PBS and incubated in 5 ml of the above medium at 37°C for 3 days. Following a total of four such sequential passages on $CV_1$ cells, the cell lysates were titrated on $CV_1$ cells, and one lysate (originating from a stock mutagenized for 3 min) was found to contain $2.5 \times 10^7$ PFU per $10^6$ cells. Virus from one of these plaques was plaque purified twice more on $CV_1$ cells; stocks were then prepared on human cells before use in subsequent experiments. Parallel passage of the original nonmutagenized stock on $CV_1$ cells resulted in less than 5 PFU per $10^6$ cells after four passages.

The variant (hr400) formed plaques with similar efficiency on HeLa, $CV_1$, BSC-1 (an established line of AGMK cells), and primary AGMK cells. This is in marked contrast to its parent (Ad2), which produces plaques $10^3$- to $10^4$-fold less efficiently on monkey cells than on human cells (Table 1). Although the plaque morphology of hr400 on HeLa, BSC-1, and AGMK cells was similar to that of Ad2$^+$ND1, hr400 on $CV_1$ cells produced minute plaques in comparison with those of Ad2$^+$ND1. Titration of hr400 on both human and monkey cells indicated that plaque formation followed one-hit kinetics. The mutation appeared to be quite stable, since there was no loss in the efficiency of plaque formation on monkey cells as compared to human cells after five passages on HeLa cells, where there was no obvious selective pressure to maintain the new phenotype.

The kinetics of growth and burst size of hr400 were compared to those of Ad2, Ad2$^+$ND1, and

Ad2-plus-SV40 infections on HeLa, $CV_1$, and primary AGMK cells (Fig. 2; Table 2). Ad2, Ad2+ND1, hr400, and Ad2 in the presence of SV40 multiplied at about the same rate and produced nearly equivalent amounts of virus (PFU) on HeLa cells. Ad2 in the presence of SV40 grew equally well on HeLa, $CV_1$, and primary AGMK cells. Ad2+ND1 and hr400 multiplied with equal efficiency on HeLa and $CV_1$ cells, but did so severalfold less efficiently on AGMK cells. Ad2 in the absence of SV40 grew approximately 1,000-fold less well on the two types of monkey cells than on human cells. In fact, the total number of infectious particles of Ad2 present in the cell culture shortly after infection (5 h) did not increase during the abortive cycle. Since some plaques were formed on Ad2-infected monkey cells, the constant level of virus throughout the abortive cycle was probably the result of inactivation with time of residual amounts of infecting virus, counterbalanced by low levels of production of new virus in at least some of the monkey cells.

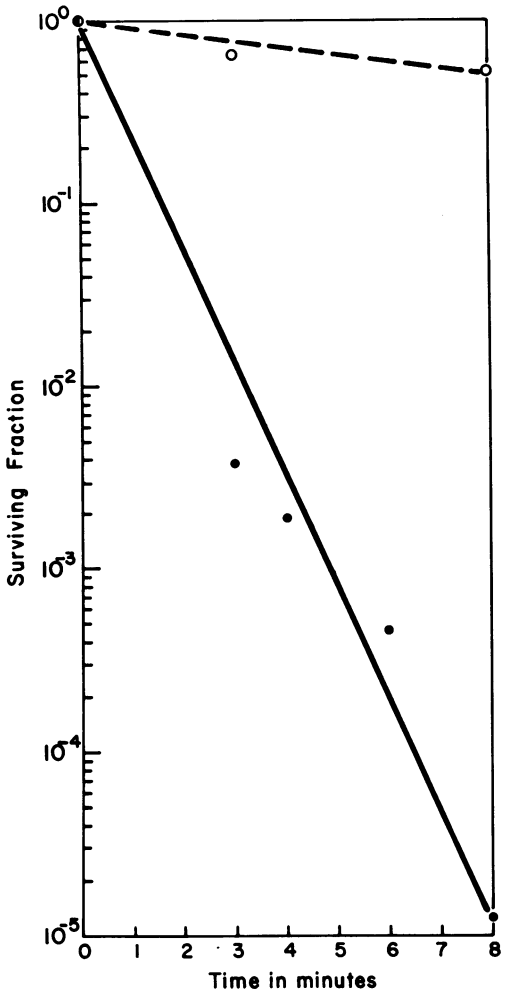


FIG. 1. *Survival curve of Ad2 after mutagenesis. At the times indicated a portion was removed from the mutagenesis reaction containing acetic acid and $NaNO_2$ (●——●) or from the control reaction containing only acetic acid (○---○), and then it was titrated on HeLa cells as described previously (6).*

The similarity in growth properties of hr400 and Ad2+ND1 on $CV_1$ cells was unexpected in view of the smaller plaque size of hr400. Perhaps hr400 has a mutation in a gene coding for a structural protein such as fiber, which may decrease the efficiency of adsorption, penetration, and/or uncoating of the virus or reduce the efficiency of lysis on $CV_1$ cells. Since plaque formation requires several cycles of infection, and the conditions for each cycle are different, a decrease in the efficiency of any of these processes could have a dramatic effect on plaque morphology without noticeably affecting the burst size or even the kinetics of growth for one round of infection.

Since the presence of a small region of the SV40 genome in SV40-Ad2 hybrids is sufficient to overcome the block to adenovirus multiplication in monkey cells (13), it is essential to show that hr400 does not contain SV40 sequences that might have been accidentally acquired during the selection process. Restriction endonuclease analysis and hybridization studies have failed to detect any (less than 60 base pairs) SV40 sequence in hr400. In addition, the restriction endonuclease analysis showed no differences between the genomes of hr400 and its parent. The details of these studies will be presented in a later communication.

TABLE 1. *Plaquing efficiency on monkey cells versus human cells*[a]

| Virus | $CV_1$ | BSC-1 | AGMK |
|---|---|---|---|
| Ad2+ND1 | 0.77 to 1.54 | 0.04 to 0.31 | 0.08 to 0.1 |
| hr400 | 0.56 to 1.0 | 0.04 to 0.25 | 0.11 to 1.0 |
| Ad2 | 0.0005 to 0.0017 | 0.0001 to 0.0004 | 0.0003 to 0.0006 |

[a] Plaquing efficiency is expressed as the ratio of number of plaques on the various types of monkey cells versus the number of plaques on HeLa cells. The numerical values indicate the range of these ratios determined from 2 to 5 experiments. Plaquing was done as described previously (6), except that in some experiments 5% fetal bovine serum was used instead of 2% gamma globulin-depleted calf serum.

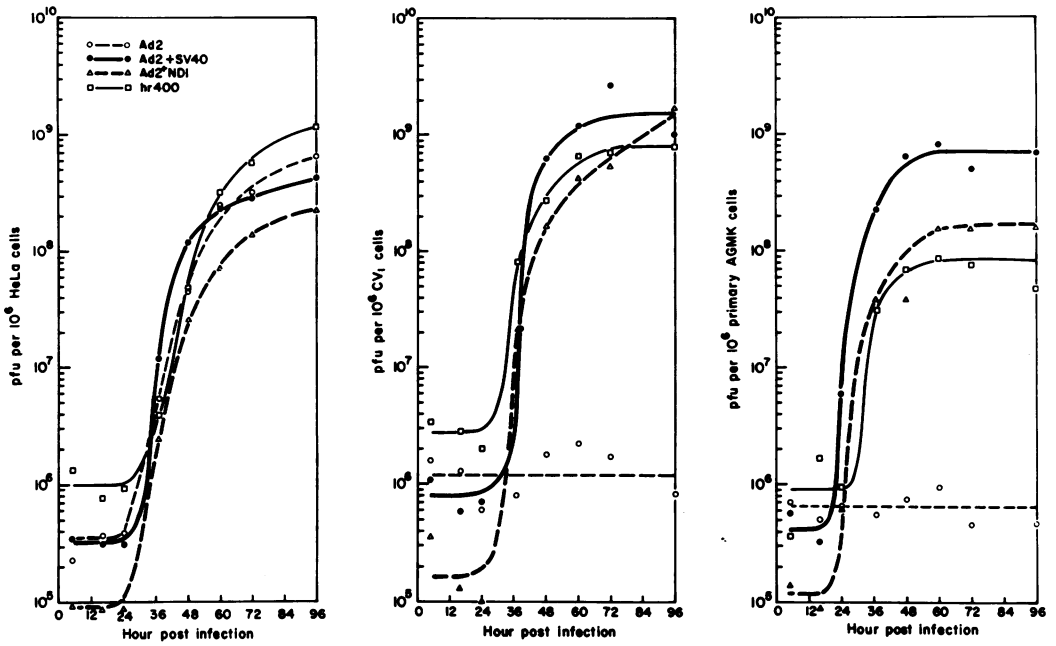


FIG. 2. *Kinetics of multiplication of several types of adenovirus on HeLa, $CV_1$, and primary AGMK cells. Confluent plates of HeLa, $CV_1$, or primary AGMK cells were passaged 14 to 18 h before infection. High-titer stock ($5 \times 10^9$ to $3 \times 10^{10}$ PFU/ml) of Ad2, hr400, or Ad2+ND1 was incubated with 100 μg of trypsin per ml for 30 min at 37°C before dilution in PBS and addition of 5 PFU per cell to the monolayer in 0.5 ml of PBS. SV40 (10 PFU per cell) was used in coinfection with Ad2 where indicated. After 1 h of adsorption, fresh medium was added. At various times after infection, the cell monolayer and medium were harvested by scraping. After the cells were opened by freezing and thawing followed by brief sonic treatment, the cell lysate was titrated on HeLa cells. SV40 does not form plaques on HeLa cells.*

TABLE 2. *Growth of several different adenovirus strains on HeLa, $CV_1$, or primary AGMK cells*[a]

| Host | Experiment | Virus | | | |
|---|---|---|---|---|---|
| | | Ad2 | hr400 | Ad2+ND1 | Ad2 + SV40 |
| HeLa | I[b] | $10.9 \times 10^8$[c] | $11.4 \times 10^8$ | $4.7 \times 10^8$ | $10.9 \times 10^8$ |
| HeLa | II | $6.6 \times 10^8$ | $11.6 \times 10^8$ | $2.3 \times 10^8$ | $4.4 \times 10^8$ |
| $CV_1$ | I | $1.5 \times 10^6$ | $2.0 \times 10^8$ | $3.2 \times 10^8$ | $6.9 \times 10^8$ |
| $CV_1$ | II | $1.2 \times 10^6$ | $8.0 \times 10^8$ | $15.0 \times 10^8$ | $15.0 \times 10^8$ |
| AGMK | I | $0.4 \times 10^5$ | $1.0 \times 10^8$ | $2.5 \times 10^8$ | $6.2 \times 10^8$ |
| AGMK | II | $6.6 \times 10^5$ | $0.8 \times 10^8$ | $1.7 \times 10^8$ | $7.0 \times 10^8$ |

[a] The procedures for infecting, harvesting, and titrating are described in the legend to Fig. 2. Cells were harvested 96 to 98 h after infection.

[b] The kinetics of growth for the different viruses on the various cell types for experiment I are shown in Fig. 2.

[c] The numerical values express the numbers of PFU per $10^6$ cells.

The map location of the mutation in hr400 is being determined, with the hope that it can be correlated with a known gene. Such a correlation should give a hint as to the biochemical events that are responsible for the abortive nature of Ad2 infection in monkey cells but that are altered in hr400-infected monkey cells so that viral multiplication is permitted. In addition, more variants of the type described are being selected, to determine if additional genes might be directly involved in the block to viral multiplication.

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