Response of Simian Virus 40-Transformed Cell Lines and Cell Hybrids to Superinfection with Simian Virus 40 and Its Deoxyribonucleic Acid

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Whereas normal human and monkey cells were susceptible both to intact simian virus 40 (SV40) and to SV40 deoxyribonucleic acid (DNA), human and monkey cells transformed by SV40 were incapable of producing infectious virus after exposure to SV40, but displayed susceptibility to SV40 DNA. On the other hand, mouse and hamster cells, either normal or SV40-transformed, were resistant both to the virus and to SV40 DNA. Hybrids between permissive and nonpermissive parental cells revealed a complex response: whereas most hybrids tested were resistant, three of them produced a small amount of infectious virus upon challenge with SV40 DNA. All were resistant to whole virus challenge. The persistence of infectious SV40 DNA in permissive and nonpermissive cells up to 96 hr after infection was ascertained by cell fusion. The decay kinetics proved to be quite different in permissive and nonpermissive cells. Adsorption of SV40 varied widely among the different cell lines. Very low adsorption of SV40 was detected in nonsusceptible cells with the exception of the mKS-BU100 cell line. A strong increase in SV40 adsorption was produced by pretreating cells with polyoma virus. In spite of this increased adsorption, the resistance displayed by SV40-transformed cells to superinfection with the virus was maintained.

Fusion of permissive normal African green monkey kidney (AGMK) cells with some non-virus-yielding simian virus 40 (SV40)-transformed cells has resulted in activation of the SV40 genome (3, 9). Further experimentation has shown that fusion of nonpermissive SV40-transformed cells with permissive SV40-transformed cells also yields virus (F. C. Jensen and H. Koprowski, 1969, *Virology* 37:687-690). These results suggest the absence of a repressor in SV40-transformed monkey and human cells, for the existing genome(s) can replicate when given the proper milieu. To verify this suggested lack of a repressor, experiments were undertaken to expose SV40-transformed cells to superinfection with intact SV40 and SV40 deoxyribonucleic acid (DNA). Normal nonpermissive cells, capable of SV40-transformation but incapable of expression of late viral functions, were similarly treated in order to clarify the mechanism of this resistance to viral replica-

tion. The response of hybrid cells, of permissive (human) and nonpermissive (mouse) parental origin, to SV40 and SV40 DNA was also investigated to see whether the addition of portions of a permissive genome could overcome the innate resistance of the mouse cell to viral replication. The accidental discovery of increased adsorption of intact SV40 to cells after treatment with polyoma virus was utilized to further delineate the mechanism of cellular resistance to whole SV40.

MATERIALS AND METHODS

Cell cultures. The cells used in the present study and their passage levels (when available) are listed in Table 1. All tissue cultures were grown in double strength Eagle's amino acids and vitamins in Earle's balanced salt solution (BSS) with 50 μ g of aureomycin per ml and 10% fetal calf serum (FCS). An exception was that of primary AGMK cells which were maintained in Earle's BSS with 0.5% lactalbumin hydrolysate (LAH), 2% FCS, and aureomycin. After infection with virus or SV40 DNA, cells were maintained in Eagle's medium which contained 2% FCS (maintenance medium).

Infection of cells with SV40. Cells to be infected with virus were seeded into 60-mm petri dishes at

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TABLE 1. *Origin of cell lines used in the present study*

Species	Cell type				
	Primary	SV40-transformed			
		No	Passage no.	Yes	Passage no.
Hamster	HEF	CHO ^a Nil-2 ^f B-1 ^b	49 74-89	F5-1 cl 2	103-111
Monkey	AGMK	CV-1	34-51	EVa cl 2A-1 cl 2B-1 cl 6-1 LLE46 A-10-2 ^g	79-97 79 69-70 107-132
Mouse	CBA-T6 MEF	LM(TK ⁻)cl 1-D		mKS-BU100 cl-1-1 ^h	
Man		WI-38 K1	27-33 19	W18Va ₂ W18Va ₂ (W) ^c WI26Va ₄ W98VaC	389-422 320-338 146-158
Man-mouse		HM ₂ P ₅ ^d HMSV ^c FAM-1 (TK ⁺) ^d FAM-1 (H ⁻) ^d 4W10 ^e 4W7 ^e 3W4 ^e 1W1 ^e VT-7			

^a Proline-dependent Chinese hamster cell line obtained from T. T. Puck.

^b Subline of BHK-21 cell line obtained from John W. Littlefield.

^c Obtained from Mary Weiss (15). The cell line LM (TK⁻) cl 1-D is referred to subsequently in this paper as cl 1-D.

^d Obtained from Howard Green (10).

^e Obtained from V. Miggiano (V. Miggiano, M. Nabholz, and W. Bodmer, 1969, Wistar Inst. Symp. Monogr., *in press*).

^f Obtained from L. Diamond (2).

^g AGMK cells transformed by Adeno-7-SV40 hybrid LLE46 virus (6).

^h Obtained from D. Dubbs (3). Abbreviations: HEF, hamster embryonic fibroblasts; MEF, mouse embryonic fibroblasts from Swiss mice; CBA-T6, mouse embryonic fibroblasts from the CBA-T6 strain of mice; AGMK, African green monkey kidney; W18Va₂(W), selected by M. Weiss for resistance to 8-azaguanine; K1, fibroblast culture from biopsy of a Lesch-Nyhan syndrome patient. Courtesy of J. Dancis, New York University Medical School. For information about all other cell lines see Koprowski et al., (9).

1×10^6 cells per dish and grown to confluence. The medium was removed, the cells were washed once in phosphate-buffered saline (PBS), and 100 plaque-forming units (PFU) per cell of SV40 was added to the dishes (total volume, approximately 1 ml). After a 2-hr incubation period at 37 C in a humidified CO₂ incubator, the virus was washed from the cells with three changes of PBS. Maintenance medium (5 ml) was added, and after 24- and 72-hr incubation periods, the cells in the petri dishes were harvested by scraping them into their own medium. The cells were disrupted

by sonic treatment (Raytheon, 100 v, 1 amp, 1 min) and frozen at -20 C until plaque test titration.

Plaque assay. For the plaque test titration, 0.2 ml of the cell extract was adsorbed to monolayers of primary AGMK cells in 60-mm petri dishes for 2 hr at 37 C. A 5-ml overlay of 1.0% agar in double strength Eagle's medium in Earle's BSS with 0.125% LAH and 7.5% FCS was then added. At 6 days, a 3-ml overlay was added; after further incubation, a 3-ml overlay, containing 1:10,000 neutral red, was

added at 10 days. The final plaque reading was from 17 to 21 days.

Purification of SV40. The procedure elaborated by K. Yoshiike and D. Axelrod (*personal communication*) was used for the purification of SV40. SV40 strain Rh 911 (13) was grown in CV-1 cells (passage no. 34 to 51) at an input multiplicity of 0.1 to 1.0 PFU/cell. When the cytopathic effect exceeded 75%, the cells were frozen and thawed in their medium three times and the cell debris separated from the supernatant fluid by centrifugation at $2,000 \times g$. The resulting pellet was disrupted by sonic oscillation (2 cycles of 1 min each) and centrifuged at $2,000 \times g$; this supernatant fluid was then reunited with the first supernatant fraction. These crude pools generally contained 10^8 PFU of SV40 per ml.

Further concentration was achieved by pelleting the virus at $69,000 \times g$ for 3 hr at 0 to 4 C. The pellet was resuspended in a small volume of maintenance medium and treated for 30 min at 37 C with 1% sodium-deoxycholate and 0.025% trypsin. FCS was added to give a final concentration of 7%, and the suspension was centrifuged. Portions of 20 ml of supernatant fluid were layered over 9 ml of 0.05 M tris(hydroxymethyl)aminomethane (Tris)-chloride buffer (pH 7.8) saturated at 15 C with KBr. After centrifugation for 3 hr at 15 to 17 C at $54,000 \times g$ in an SW-25.1 head of a Spinco L centrifuge, the lower of two bands which formed in the KBr was removed and filtered on a Sephadex G-100 column (30 by 2 cm) which was equilibrated with 0.05 M Tris acetate, pH 7.4.

^{32}P labeling of SV40. Ten hours after infection of CV-1 or AGMK cells with 50 to 100 PFU/cell of SV40, the medium was removed and new phosphate-free maintenance medium, containing 10 μC of carrier-free ^{32}P (Schwartz BioResearch Inc., Orangeburg, N.Y.), was added. The cultures were harvested when all of the cells showed virus-specific cytopathology. The virus was purified, as previously described.

Isolation of SV40 DNA. The procedure used was similar to that described by Winocour (16). The virus-containing fractions of the eluant were pooled and solid NaCl was added until the solution was 1 M. An equal volume of 9 parts 80% phenol and 1 part 2 M sodium trichloroacetate and 0.01 M ethylenediamine-tetraacetic acid (EDTA), pH 7.1, was added and shaken for 15 min at room temperature. The mixture was centrifuged at $8,000 \times g$ for 15 min in a Servall centrifuge. After removal of the aqueous phase, the interphase precipitate was extracted two times with $\frac{1}{6}$ of the original volume of 0.05 M Tris-chloride (pH 8.2) and 1 M NaCl. The aqueous phase was pooled and again extracted with 80% phenol at room temperature. Four extractions with ether removed the remaining phenol from the aqueous phase, and the ether was eliminated by bubbling nitrogen through the solution. The DNA was precipitated with ethyl alcohol and collected after centrifugation in $0.1 \times \text{SSC}$ ($\text{SSC} = 0.15 \text{ M NaCl}, 0.015 \text{ M sodium citrate}$). The concentration of DNA was estimated spectrophotometrically, and its infectivity was determined by titration on primary AGMK cells by the method of McCutchan and Pagano (11). The titer was 10^4 PFU/ μg of DNA.

To eliminate contamination of the DNA with intact virus, controls with deoxyribonuclease-treated DNA were performed; prior to adsorption DNA was digested with deoxyribonuclease (20 $\mu\text{g}/\text{ml}$) in presence of 0.003 M Mg^{2+} for 30 min at 37 C. No infectivity was detected in these samples.

Infection of cells with SV40 DNA. A confluent monolayer in a 60-mm petri dish was washed with PBS without Ca^{2+} and Mg^{2+} ; SV40 DNA (3 μg) and 300 μg DEAE Dextran (Pharmacia, Uppsala, Sweden; lot no. 4763) in 0.6 ml PBS without Ca^{2+} and Mg^{2+} were layered on top of the cell monolayer. After incubation for 15 min at room temperature, the inoculum was discarded and the cells washed three times with PBS. After the addition of 5 ml of maintenance medium, the cells were incubated for 72 hr at 37 C in a CO_2 incubator, to allow for the completion of a full replicative cycle of SV40. The cells were then scraped into their own medium and disrupted by sonic oscillation prior to plaque assay on AGMK monolayers. To ensure that resulting infectivity was due to replicated virus and not remaining input DNA, the cell homogenate was treated with deoxyribonuclease, as above.

Cell fusion techniques. The technique of cell fusion was essentially the same as previously described (8). The cells were harvested in trypsin-EDTA at various time intervals after exposure to SV40 DNA and incubated at 37 C for 30 min with deoxyribonuclease in order to eliminate the possibility of any contaminating extracellular DNA. An equal number of these cells and AGMK cells were then incubated together in the presence of β -propiolactone-inactivated Sendai virus. The resultant fused-cell population was seeded into milk dilution bottles and allowed to grow for 1 week. The cells were then scraped into their medium and disrupted by sonic oscillation, and the extract was assayed for infectious SV40.

Adsorption of ^{32}P -labeled SV40 to cell monolayers. Cell monolayers in 60-mm petri dishes containing 2×10^6 to 3×10^6 cells were washed once with PBS and subsequently 50 to 100 PFU/cell was added in a volume of 1 ml of maintenance medium. After 2 hr of adsorption at 37 C the supernatant fluid was removed, and the remaining cell monolayers were washed three times with PBS at room temperature; 1 ml of PBS containing 10 mg of anti-SV40 gamma globulin was added, and the cells were incubated for 1 hr at 37 C. After removal of the antibody the cells were again washed three times with PBS and finally trypsinized. The number of cells was determined by cell count, and the cells were sedimented by centrifugation at $350 \times g$. The pellet was resuspended in 0.5 ml of water, and cells were disrupted by ultrasonic treatment. Portions of the homogenate were transferred to glass fiber discs, and, after drying, the radioactivity was determined by liquid scintillation counting.

Anti-SV40 gamma globulin. Anti-SV40 serum was prepared by intravenous injection of purified SV40 suspension into New Zealand white rabbits. The gamma globulin fraction was isolated and titrated for SV40 neutralizing activity; 1 mg of gamma globulin was found to neutralize 10^6 PFU of SV40.

Pretreatment of cells with polyoma virus. Of crude preparation of sonically treated mouse embryo fibroblasts (MEF) homogenate containing polyoma virus at the titer of 10^8 PFU/ml, 1 ml was added to each petri dish and allowed to adsorb for 90 min at 25 C. The supernatant fraction was removed, and the cell sheet was washed with PBS prior to infection with SV40 virus. As control, MEF homogenate was obtained by freezing and thawing uninfected MEF cells in their own medium and subsequently applying ultrasonic treatment for 2 min at 4 C.

RESULTS

Resistance of SV40-transformed cells to challenge with SV40. The results of infectivity assays (Table 2) indicate that an increase in virus titer between 24 and 72 hr after infection was observed in only three of the cell lines tested. Two of these cultures were normal African green monkey kidney (CV-1) and normal human (WI-38) cells. The third culture capable of supporting viral replication was an AGMK line transformed by the adeno-7-SV40 "hybrid" virus, LLE46. In all the remaining cultures, no increase in virus titer was observed between the 24-hr and 72-hr harvest. The residual infectivity present was probably caused by the large amount of adsorbed "background" SV40, as it could be reduced by treatment of the infected cells after the period of absorption with anti-SV40 gamma globulin. In addition to the SV40-transformed cells, the six man-mouse hybrid lines also were unable to produce infectious virus.

Adsorption of SV40 cells. To test whether the resistance of cells to infection or superinfection with SV40 was a reflection of their inability to adsorb the virus, 32 P-labeled SV40 was adsorbed to different cells, and the degree of adsorption was determined. As shown in Table 3, the adsorption of SV40 varied greatly among the different cells. By using a multiplicity of infection of 50 to 100 PFU/cell, primary AGMK cells adsorbed 50% of input virus, whereas a lower amount, 12%, was adsorbed by the cells of a secondary passage of AGMK. The established AGMK cell line CV-1 and cells of an AGMK line transformed by the adeno-7-SV40 hybrid (LLE46 A-10-2) adsorbed less virus. Markedly less virus was adsorbed by EVa cl 2A-1 cells and hamster and mouse cells, whether normal or SV40-transformed, with the exception of the mouse cell line mKS-BU100, which adsorbed a large amount of virus. In a different study it was observed that pretreatment of cells with polyoma virus suspension enhanced the adsorption of SV40 (Table 3). Therefore, attempts were made to find out whether, under the condition of increased adsorption, the in-

TABLE 2. *Production of infectious virus after exposure of cells to SV40*

Origin	Cell type		SV40 yield in log PFU/ 10^6 cells after exposure to SV40	
	SV40-transformed		24 hr	72 hr
	No	Yes		
Mouse	CBA-T6 cl 1-D		4.3	4.2
			3.8	3.8
		mKS-BU100	4.1	4.0
Hamster	HEF B-1 CHO Nil-2		4.0	4.1
			4.1	3.9
			3.8	3.8
		F-5-1	4.2	4.2
Monkey	CV-1		3.9 ^a	5.9
			3.8	3.8
		EVa cl 2A-1 LLE46 A-10-2	3.7	5.4
Man	WI-38		3.9	4.8
			3.8	3.8
		W18Va ₂	3.8	4.1
		W126Va ₄	4.4	4.6
Man-mouse	HM:P ₅		3.8	3.9
			3.9	3.9
		4W10	4.7	4.7
		4W7	4.3	3.9
		3W4	4.3	4.0
		1W1	4.1	4.1

^a Values in italics are for cultures showing an increase in virus titer between 24 and 72 hr after infection was observed.

bility of EVa cl 2A-1 to support SV40 replication could be overcome.

These cells and CV-1 cells were pretreated with polyoma virus and with noninfected MEF extract and then challenged with SV40 (Table 4).

Pretreatment with polyoma virus, but not with MEF homogenate, resulted in higher residual infectivity of the EVa cl 2A-1 cells at 24 hr after exposure to SV40. As in the case of nonpretreated cells, this larger concentration of "background" SV40 remained constant at 72 hr after exposure. Preincubation with polyoma virus also markedly stimulated adsorption of SV40 to CV-1 cells, but the increase in the yield of virus in pretreated and untreated cells was similar ($10^{4.9}$ to $10^{6.1}$ PFU/ 10^6 untreated cells and $10^{6.2}$ to $10^{7.2}$ PFU/ 10^6 cells after polyoma treatment).

Susceptibility of SV40-transformed cells to challenge with SV40 DNA. Cells were exposed to SV40 DNA and assayed for presence of infectious virus 72 hr later (Table 5). The results indicate that challenge of normal and SV40-transformed mouse and hamster cells with SV40 DNA did not lead to the production of SV40. On the other hand, both the normal and the transformed cells of

TABLE 3. Adsorption of SV40 to pretreated cells

Cell type	Susceptible to SV40	Susceptible to SV40 DNA	Per cent of input of ³² P-labeled SV40 adsorbed on cells		
			Control	Pretreated with MEF homogenate	Pretreated with polyoma virus
AGMK (primary)	+	+	49.8 ± 3.3 (2) ^a		48 ± 6.0 (2)
(secondary)	+	+	12.1 ± 2.1 (3)		NT ^b
CV-1	+	+	2.1 ± 0.7 (5)	2 (1)	4.3 ± 0.9 (2)
LLE46 A-10-2	+	+	3.2 ± 0.5 (5)		6.5 ± 0.9 (2)
EVa cl 2A-1	—	+	0.2 (7)		2.4 ± 0.5 (3)
cl 1-D	—	—	0.1 (3)		0.5 (2)
mKS-BU100	—	—	35.6 ± 4.6 (5)	36 (1)	50 (1)
F-5-1	—	—	0.4 (2)	0.4 (2)	1.6 (2)

^a Numbers in parentheses indicate number of experiments performed.^b Not tested.

TABLE 4. Effect of treatment with polyoma virus on SV40 infection of CV-1 and SV40-transformed cells

Cell type	Time of harvest after SV40 infection (hr)	SV40 yield in log PFU/10 ⁶ cells		
		Control	MEF homogenate	Polyoma virus
CV-1	24	4.9	4.9	6.0
	72	6.1	6.3	7.2
EVa cl 2A-1	24	4.9	5.0	6.6
	72	4.9	5.1	6.8

human or monkey origin produced infectious SV40 upon exposure to SV40 DNA.

Penetration of SV40 DNA into nonpermissive cells. To determine whether the resistance of mouse and hamster cells to infection with SV40 DNA is conditioned by the inability of the DNA either to penetrate the cells or to persist long enough to be able to initiate synthesis of the virus, cells (Table 6) were exposed to SV40 DNA as before and 24 hr later were fused with susceptible AGMK cells. This method was shown by Kit et al. (7) to be a sensitive technique for demonstration of the presence of intracellular SV40 genome. Heterokaryon cultures derived from fusion of AGMK cells with both nonpermissive and permissive cells produced infectious SV40 when tested for virus production 7 days after fusion (Table 6).

Survival of SV40 DNA within transformed and nonpermissive cells. The time of survival of SV40 DNA within a nonpermissive mouse line (cl 1-D) and an SV40-transformed human cell line W18Va₂ (W) was investigated in the following experiment. Cells of the two cultures were exposed to SV40 DNA and fused with equal numbers of AGMK

TABLE 5. Production of infectious virus after exposure of cells to SV40 DNA

Cell type			SV40 yield log PFU/10 ⁶ cells 72 hr after exposure to SV40 DNA
Origin	SV40-transformed		
	No	Yes	
Mouse	MEF CBA-T6 cl 1-D	mKS-BU100	0.0
			0.0
			0.0
			0.0
Hamster	HEF B-1 CHO Nil-2	F-5-1	0.0
			0.0
			0.0
			0.0
			0.0
Monkey	CV-1	EVa cl 2A-1 cl 2B-1 cl 6-1 LLE46 A-10-2	4.4
			2.1
			3.2
			3.7
			4.0
Man	WI-38 HeLa	W18Va ₂ W18Va ₂ (W) WI26Va ₄ W98VaC	3.5
			2.0
			3.2
			2.8
			3.6
			3.9

cells at 24, 48, 72, and 96 hr after infection. After 1 week of incubation, the heterokaryon cultures were sonically treated and the undiluted cell extracts were tested for the presence of infectious SV40 in tubes of primary AGMK cells.

As shown in Fig. 1, at 24 hr after exposure to SV40 DNA, the degree of cytopathology produced by cell extracts from the cl 1-D × AGMK cultures was the same as that from heterokaryon

cells of W18Va₂ (W) × AGMK cultures. However, when fusion took place 96 hr after exposure to SV40 DNA, the cytopathic effect produced by cl 1-D × AGMK cell extracts was barely detectable, whereas cell extracts of W18Va₂ × AGMK heterokaryons produced complete cytopathic effect.

Susceptibility of man-mouse hybrid cells to SV40 DNA. To investigate susceptibility of hybrid cell lines, produced between permissive and non-permissive cells, to SV40 DNA, the hybrids and parental cultures listed in Table 7 were exposed to SV40 DNA. The cell extracts were assayed for presence of infectious virus 72 hr later.

Infectious SV40 was produced by the two parental human lines, the SV40-transformed W18Va₂ (W) and the normal WI-38 (Table 7). Cells of three of the nine man-mouse hybrid cultures produced infectious SV40. The VT-7 line produced less than 10 PFU/10⁶ cells in two out of eight experiments performed; cells of another positive line, HM₂P₅, produced small amounts of virus detected in undiluted cell extracts in the only two experiments carried out. 4W10 was positive in one out of two experiments performed.

To ascertain that infectivity of the cells of the three man-mouse hybrids was not due to residual input DNA but to newly synthesized complete virus, cell extracts obtained from the three hybrid cultures after exposure to SV40 DNA were treated with deoxyribonuclease and anti-SV40 gamma globulin before infecting the AGMK cells. The results showed that treatment with deoxyribonuclease did not affect infectivity of the preparations, whereas treatment with anti-SV40 gamma

TABLE 7. Exposure of man-mouse hybrid cells to SV40 DNA

Origin	Cell type	Approximate no. of human chromosomes at time of experiment	Cell surface antigen	SV40 virus yield log PFU/10 ⁶ cells
Man	W18Va ₂ (W) ^a WI-38 ^b	74-84 46	Hu ^c Hu	2.0 3.6
Mouse	cl 1-D ^b CBA-T6 ^a	0 0	Mo Mo	0 0
Man-mouse	VT-7 HM ₂ P ₅ 4W10 HMSV ^d FAM-1 (TK ⁺) FAM-1 (H ⁻) ^d 4W7 3W4 1W1	8-15 9-12 8-12 2-5 2 0 5-9 3-6 1-4	Hu-Mo Hu-Mo Hu-Mo Hu-Mo Hu-Mo Mo Hu-Mo Hu-Mo Hu-Mo	+ ^e + + 0 0 0 0 0 0 0

^a Parental cells of VT-7

^b Parental cells of HM₂P₅

^c Hu, human; Mo, mouse.

^d Human parental cell not tested, mouse parental cell—cl 1-D. FAM-1 (H⁻) line is the FAM-1 (TK⁺) hybrid line in which loss of all human type chromosomes was observed [see Matsuya, Green, and Basilico (10)].

^e + = SV40 virus isolated in some experiments.

globulin did inactivate the virus. This seemed to indicate that VT-7, HM₂P₅, and 4W10 cells were capable of synthesizing low levels of infectious virus after their exposure to SV40 DNA.

DISCUSSION

Data on challenge of cell lines transformed by an oncogenic DNA virus have been obtained primarily from work with polyoma virus. Wino-cour and Sachs (17) observed a resistance of uncloned cultures derived from polyoma-induced parotid tumors of mice to challenge with polyoma virus, and showed that "single cell" clone populations derived from the same tumors were susceptible to superinfection. Vogt and Dulbecco (14) selected, in the course of transformation by polyoma virus, mouse cell lines which gave rise to populations resistant to superinfection. Conversely, results obtained by Hellström et al. (5) indicated that five out of six cell lines derived from polyoma-induced mouse tumors were susceptible to superinfection. Resistance of one cell line derived from mouse cultures originally transformed in vitro and carried as tumor transplant in vivo could be overcome by increasing the dose of the challenge virus. Further studies of Hellström (4) indicated marked variability in

TABLE 6. Production of infectious SV40 in cultures of cells infected with SV40 DNA and fused with AGMK cells 24 hr later^a

Origin	Cell type	SV40 yield log PFU/ml 7 days after fusion
Mouse	CBA-T6 ^b cl 1-D ^b	3.8 3.5
Monkey	AGMK	4.8
Man	K1 W18Va ₂	3.8 3.3
Man-mouse	VT-7 HMSV ^b	2.6 3.7

^a SV40 DNA-infected cells were pretreated with deoxyribonuclease prior to fusion.

^b Cells which did not produce infectious SV40 virus upon exposure to SV40DNA.

susceptibility to polyoma virus challenge of sublines obtained by single cell cloning from the same polyoma tumor.

In contrast, in this study, cells of lines derived from permissive human and AGMK cultures transformed by infectious SV40 were found to be uniformly incapable of producing infectious virus after superinfection with SV40. At first glance, resistance to superinfection seemed to be related to the decreased capacity of the transformed cells to adsorb the challenge virus. However, even though pretreatment with polyoma virus increased SV40 adsorption 10-fold, the transformed cells still remained incapable of supporting SV40 replication. In addition, cells from a nonpermissive SV40-transformed mouse line, mKS-BU100, adsorbed approximately as much SV40 as did fully susceptible AGMK cells (Table 3).

The culture LLE-46 A-10-2, transformed by the adeno-7-SV40 "hybrid" virus, was capable of producing infectious virus after SV40 challenge (6). Cells of this line differ in many respects from those of other AGMK lines transformed by SV40 alone. For instance, LLE-46 A-10-2 cells, in contrast to EVa cl 2A-1 cells, show an only slightly enhanced growth capacity over AGMK cells; their growth is also much more inhibited by the parental AGMK and rabbit lens cells (H. Eagle, F. Foley, H. Koprowski, and H. Levine, *in preparation*). In addition, after ruthenium red staining, these cells are shown by electron microscopy to have a considerably less undulated surface area than do the EVa cl 2A-1 cells (A. Vorbrodt and H. Koprowski, *J. Nat. Cancer Inst.*, *in press*). Finally, no SV40 transplantation antigen has been detected in these cells (6). Whether any one or a combination of these properties accounts for the susceptibility of LLE-46 A-10-2 cells to superinfection by SV40 is difficult to state at present.

In contrast to their resistance to challenge with SV40, all human and AGMK transformants were capable, after exposure to viral DNA, of supporting synthesis of infectious SV40. This confirms the observation (F. C. Jensen and H. Koprowski, *Virology* 37:687-690.) that these cells are able to synthesize virus after fusion with transformed nonpermissive cells which contain the viral genome but are themselves incapable of supporting completion of viral synthesis. These results also suggest that transformation of human and AGMK cells by SV40 does not involve the formation of a repressor molecule similar to the one present in lysogenic bacteria (12).

Normal and SV40-transformed mouse and hamster cells were resistant to challenge with SV40 even though cells of one line, mKS-BU100, were capable of adsorbing almost as much virus

as were fully susceptible AGMK cells (Table 3). Mouse and hamster cells were unable to replicate SV40 after exposure to viral DNA, although it was interesting that some infectious SV40 DNA could still be detected in these abortively infected cells for 96 hr after exposure to the viral DNA (Fig. 1). This result confirms observations made by Kit et al. (7) who found that infectious SV40 DNA could be extracted from mouse kidney cells up to 50 hr after exposure to SV40. Thus, the continuing presence of SV40 DNA, even though in decreasing amounts, does not seem to be of importance per se in determining the ability of the cell to support synthesis of complete virus. Observations made by zur Hausen (*personal communication*) on the persistence but nonreplication of adenovirus type 12 genomes for at least 96 hr in nonpermissive hamster cells exposed to the adenovirus are directly similar to our results with SV40.

Inability of both normal and SV40-transformed nonpermissive cell types to support synthesis of the virus after exposure to viral DNA makes it difficult to distinguish between "repressor" produced in these cells after transformation by SV40 (1) and the "natural" resistance displayed by the nontransformed cells, the latter probably being related to their species origin. It is perhaps more probable that these cells lack a factor, or several factors, necessary for complete SV40 replication and which may have been supplied in the man-mouse hybrids by the rather small number of human chromosomes on a background of complete genotype of the mouse. The facility with which the viral genome can be rescued from SV40-transformed mouse cells after fusion with either normal (8) or SV40-transformed permissive cells (Jensen and Koprowski, 1969,

FIG. 1. Persistence of the SV40 genome in nonpermissive mouse cells cl 1-D and SV40-transformed human W18Va₂ (W) cells after their exposure to SV40 DNA. The per cent cytopathic effect refers to the number of AGMK cells showing vacuolization per total number of cells.

Virology 37:678-690) also indicates that a factor necessary for viral replication is missing in the nonpermissive transformed cells. Finally, the simultaneous appearance of viral coat protein in permissive and nonpermissive nuclei within the same heterokaryocyte after SV40 infection (Z. Steplewski and H. Koprowski, 1969, Exp. Cell Res., *in press*) also argues against the presence of a specific repressor.

The susceptibility or resistance of man-mouse hybrid cells to SV40 DNA presents a problem in itself. The VT-7, HM₂P₅, and 4W10 hybrid lines were capable of producing small amounts of SV40 after exposure to SV40 DNA and contained the largest number of human chromosomes of all hybrids tested (Table 7).

However, VT-7 is a hybrid between a mouse cell and the SV40-transformed W18Va₂ (W) human cell. A certain fraction of VT-7 cells contains T-antigen (15), indicating that the SV40 genome present in the human parental cell may have been carried through to some of the hybrid cells. Whether production of very small amounts of SV40 by VT-7 cells is the result of "activation" of the SV40 genome by the incoming DNA or whether it represents virus synthesized by the incoming DNA only is impossible to say at present. If cloning attempts with the VT-7 line result in two types of cell populations, one containing only T-positive cells and the other only T-negative, then challenge of these two cell types with SV40 DNA may throw some light on this problem.

Reservations concerning the presence of the integrated viral genome do not apply to the HM₂P₅ and 4W10 cells, which are hybrids between mouse cells and nontransformed normal human cells. Thus, in this case, as well as possibly in the case of VT-7 cells, the human parental genotype may have transmitted to the hybrid cell a limited capability to support synthesis of SV40 after exposure to viral DNA. Regardless of the interpretation of the data offered at present, it is impossible to state which group of chromosomes in permissive cells determines susceptibility to SV40 infection. Availability of hybrid cells which are better characterized genetically may ultimately lead to the solution of this problem.

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