Susceptibility of Human Cell Strains to Transformation by Simian Virus 40 and Simian Virus 40 Deoxyribonucleic Acid

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Marked differences were found in the susceptibility of human fibroblasts to transformation by simian virus 40 (SV40). Highly susceptible cell strains were derived from patients with diseases associated with chromosomal abnormalities and a high incidence of tumors. In the present study, SV40 transformation-susceptible cell strains were not found to have a generalized increase in viral sensitivity. The differences in transformation frequency among cell strains with whole virus are eliminated by the use of isolated SV40 deoxyribonucleic acid, suggesting that the relative resistance of most cell strains to transformation by whole virus is due to a block at an early step in infection.

Quantitative methods for studying transformation of human diploid fibroblasts by the oncogenic virus, simian virus 40 (SV40), were previously described (11). Measuring the transformation frequency of a variety of cell strains, Todaro et al. discovered a highly susceptible group derived from patients with Fanconi's anemia (11) and Down's syndrome (12). The fact that these diseases are associated with chromosomal abnormalities has led to the speculation that cellular replication errors in these cells might facilitate the integration of SV40 into the host genome and lead to a heightened susceptibility to transformation.

In a previous report, it was shown that, for any given cell strain, the number of cells that went on to become transformed was proportional to the number of cells that produced the SV40-specific T antigen in the acute infection. These findings suggested that differences in cellular susceptibility to transformation involved steps before or associated with SV40 T antigen induction (1). In the present report, some early events in SV40 infection were examined to gain a better understanding of the step or steps responsible for the marked differences in susceptibility of human fibroblasts to transformation by SV40.

MATERIALS AND METHODS

Cell culture. Dulbecco's modification of Eagle's medium supplemented with 10% calf serum (Colorado Serum Co.) was used for growing cells. Cultures were

maintained in 50-mm plastic petri dishes and were subcultured by using 0.1% trypsin in phosphate-buffered saline.

Cells. Human fibroblast strains were derived from embryo lung, newborn foreskin, and adult skin and included cells from a number of patients with genetic diseases. Many of the cells used in these studies were grouped into three general categories of transformation sensitivity, i.e., susceptible, normal, and resistant based on their relative susceptibility to transformation by intact virus (1). The highly sensitive group included cell strains from patients with Fanconi's anemia and Down's syndrome; included in the resistant category were many human embryo fibroblast strains. Human cells were used within their first 20 generations in culture. Primary African green monkey kidney (AGMK) cells were obtained from Microbiological Associates, Bethesda, Md.

Virus. For experiments with whole virus, a single pool of plaque-purified SV40 small-plaque virus (SV-S; reference 10) titering 10^9 plaque-forming units (PFU) per ml on AGMK cells was used. It was grown under conditions which minimize the number of "defective" SV40 particles (14). Poliovirus, type 1 (Mahoney strain), titering 5×10^8 PFU/ml on AGMK cells, was obtained from K. Takemoto [National Institutes of Health (NIH)]. Vesicular stomatitis virus (VSV), titering 5×10^8 PFU/ml on AGMK cells, was supplied by S. Baron (NIH). Herpes simplex virus, titering 3×10^7 PFU/ml on human embryonic kidney (HEK), was supplied by J. Rose (NIH). Human adenovirus 7 (Ad7), strain E46⁻, and Ad7-SV40 hybrid virus (strain E46⁺) were obtained from W. Rowe (NIH).

Viral deoxyribonucleic acid (DNA). Small-plaque SV40 DNA I (closed circular form) with a titer of 10^5 PFU per μg was supplied by M. Martin (NIH). It was extracted with a mixture of chloroform and isoamyl alcohol in the presence of sodium dodecyl sulfate and sodium perchlorate and purified by equilibrium density centrifugation in cesium chloride (5).

Plaque assays. Human cell monolayers were washed with serum-free medium and then infected with 0.5 ml of appropriately diluted virus for 1 hr at 37 C. Virus was removed, and the cultures were overlaid with 5 ml of Eagle's medium containing 0.9% (w/v) Noble Agar (Difco). Another overlay containing 0.5% neutral red was added at 1 day for poliovirus and VSV and at 3 days for herpes simplex. Plaques were scored at 3 days for polio and VSV and at 5 days for herpes simplex.

SV40 transformation assay. Logarithmically growing cells were inoculated with 0.5 ml of virus (multiplicity of infection, 300 PFU/cell) and incubated at 37 C for 3 hr with frequent gentle agitation. Cultures were transferred to new petri dishes within 24 hr of infection at 10^4 and 5×10^4 cells per plate. SV40-infected cultures were continually maintained in the presence of 0.5% bovine SV40 antiserum (BBL), a concentration which neutralized between 10^5 and 10^6 plaque PFU of SV40 in 1 hr at 37 C. Transformed colonies were scored at 24 days by methods previously described (1). The transformation frequency for a particular cell strain corresponded to the total number of transformants obtained per total number of cells inoculated (corrected by a factor accounting for plating efficiency). The plating efficiency of each SV40-infected fibroblast strain tested was similar (60 to 85% when 5×10^4 cells were inoculated).

Transformation assays with SV40 DNA. Diethylaminoethyl-dextran (DEAE-D, molecular weight $>2 \times 10^6$) was shown to greatly enhance the infectivity of DNA extracted from SV40 (6), and the conditions for infection and transformation of human cells with SV40 DNA were previously described (2). For DNA infection, dividing cultures were washed twice and inoculated with 0.2 ml of Earle's balanced salt solution without sodium bicarbonate containing 0.04 to 0.4 μg of SV40 DNA I and 60 μg of DEAE-D (Pharmacia, Inc.). After incubation with DNA at room temperature for 30 min, the cells were washed twice and subcultured for the transformation assay. The transformation frequency was scored at 30 days.

SV40 T-antigen assay. SV40- and SV40 DNA-infected cells were transferred within 24 hr of infection to new petri dishes containing cover slips. SV40 T antigen-containing cells were stained at appropriate times by using the indirect fluorescent-antibody method (8). The cells were continually maintained in the presence of SV40 antiserum before T-antigen staining.

Adenovirus-SV40 hybrid infection of human cells. Cell cultures were infected with 0.3 ml of serum-free medium containing serial 10-fold dilutions of E46⁻, E46⁺, or SV40 stocks. After incubation at 37 C for 3 hr, the virus was removed, and cells in medium containing SV40 and adenovirus antiserum were trans-

ferred to petri dishes with cover slips. At 48 hr, cells on cover slips were stained either for SV40 T antigen or for adenovirus T antigen. The latter was stained by the indirect fluorescent-antibody method by using hamster adenovirus T antibody supplied by W. Rowe (NIH) and fluorescein-conjugated goat anti-hamster globulin.

Virus adsorption studies. Thymidine-methyl- ^3H -SV40 was supplied by M. Martin (NIH). It was purified by banding in cesium chloride (5) and had a specific activity of 10^5 counts per min per μg of DNA. For virus adsorption experiments, 3×10^6 cells per 100-mm petri dish were exposed to 1.0 ml of serum-free medium containing labeled virus (multiplicity of infection, 1,000 PFU/cell) for 3 hr at 37 C. The virus remaining in the supernatant was pooled in three washes (total volume, 5 ml) and precipitated with 5% (v/v) trichloroacetic acid by methods previously described (7). The cells were scraped and pooled in three washes totaling 5 ml and precipitated in 5% trichloroacetic acid. Samples were passed onto membrane filters (pore size, 0.45 μm ; Millipore Corp.), washed twice with 5% trichloroacetic acid, and placed into vials containing 10 ml of Bray's solution for counting in a Packard Tri-Carb liquid scintillation counter. Bare petri dishes were exposed to an identical inoculum of labeled virus and treated as above.

RESULTS

Comparison between SV40 T-antigen induction by whole virus and SV40 DNA. It has been shown for both human (1) and mouse cells (4) that the transformation frequency is directly proportional to the amount of SV40 added. The number of transformants obtained is also related to the number of T antigen-positive cells produced in the acute infection; those cell strains with the highest fraction of T antigen-positive cells show the highest rates of transformation (1). These findings suggest that the differences in the transformation susceptibility of human fibroblast cell strains are due to differences in virus-cell interaction at steps before or including those necessary for T-antigen production.

The ability to infect human cells with the DNA isolated from SV40 (2) provided a direct method of testing whether the virus protein coat or the viral DNA itself was responsible for the large differences in T-antigen production between cell strains. The time course of infection of human cells with whole virus and with viral DNA was compared for a transformation-susceptible (LS) and a normal (CM) human cell strain. Since there appears to be a plateau for T-antigen production and for transformation in human cells at high SV40 DNA concentrations (2), the dose response curves for T antigen were first compared in the normal and susceptible prototype cells. With each cell strain, T-antigen levels plateaued at 2 to 3% positives for DNA concentrations above 2.0

$\mu\text{g/ml}$. The amount of SV40 DNA used in subsequent experiments ($0.5 \mu\text{g}$ of DNA/ml) yielded T-antigen levels in the linear part of the dose response curve. With both whole virus and viral DNA, the peak in T-antigen production occurred at 48 to 72 hr (Fig. 1). With whole virus, the fraction of T-antigen positives was seven times higher in strain LS than in the normal strain CM. With SV40 DNA I, however, there was no difference between the peak values for T antigen in the two cultures.

Many different human cell strains were compared for sensitivity to T-antigen induction by whole virus and viral DNA (Table 1). The susceptible group included cells from patients with Fanconi's anemia (strains AM, BL, and JV) and Down's syndrome (strain 163). The percentage of T antigen-containing cells in response to whole virus ranged from 0.1 to 0.3% in five resistant to as high as 28% in the six susceptible strains. The normal group showed intermediate T-antigen levels of 0.9 to 2.3%. These results confirmed those of earlier studies (1). In contrast, SV40 DNA I induced similar T-antigen levels (0.3 to 0.6%) in parallel cultures of each of the cell strains tested.

Transformation of human cells with SV40 DNA.

The studies above showed that the marked differences in the magnitude of T-antigen production among human cell strains exposed to whole virus were eliminated when these same cells were exposed to isolated SV40 DNA. It was necessary to establish whether the differences in cell suscep-

TABLE 1. T-antigen induction in human cells by SV40 and isolated SV40 DNA

Class	Cell strain	Percentage of T antigen-containing cells using ^a	
		Whole virus	SV40 DNA I
Susceptible	FR	22.0	0.4
	LS	15.2	0.4
	BL	12.3	0.5
	AM	28.0	0.3
	JV	16.2	0.3
	163	11.2	0.5
Normal	CM	2.1	0.6
	BD	2.3	0.3
	GS	1.8	0.4
	8486	1.7	0.7
	TF	0.9	0.4
Resistant	M413	0.2	0.4
	M439	0.1	0.3
	C172	0.3	0.6
	MB	0.3	0.3
	RK	0.1	0.6

^a Parallel cultures of each cell strain were infected with SV40 (300 PFU/cell) or SV40 DNA I (0.02 PFU/cell), transferred to petri dishes containing cover slips within 24 hr, and stained for T antigen at 72 hr. Results are the average of two experiments for each cell strain.

FIG. 1. Dividing cultures of a susceptible cell strain, LS (●), and a normal cell strain, CM (△), were infected with either (i) intact SV40 virus (300 PFU/cell) or (ii) SV40 DNA I (0.02 PFU/cell). The cells were transferred after infection to new petri dishes with cover slips in the presence of SV40 antiserum. The percentage of T antigen-containing cells was scored at various times.

tibility to transformation by whole virus could be eliminated by the use of SV40 DNA.

The transforming activities of SV-S DNA I and whole virus were compared in several different fibroblast cultures (Table 2). With whole virus (multiplicity of infection, 300 PFU/cell), the level of transformation in the most susceptible strain AM was 1,800 times that observed in the most resistant strain RK. In contrast, there was no difference in the sensitivity of these same cells to transformation by infectious SV40 DNA. With DNA, the number of transformants obtained was similar for each cell strain tested (4 to 7 colonies per 5×10^5 infected cells). The level of transformation with DNA was low. However, when whole virus and DNA were compared on the basis of transformed colonies per infectious unit, the DNA was more efficient. With whole virus, to produce one transformant in the most susceptible strain, AM, approximately 7×10^4 PFU were required. At the same time, over 10^8 PFU of whole virus were required per transformed colony in the most resistant cell strain. The SV40 viral

TABLE 2. Transformation of human cells by SV40 and SV40 DNA^a

Class	No. of transformants produced by	
	Whole virus	DNA
Susceptible		
FR	1,550	4
LS	1,080	7
AM	1,800	6
Normal		
MK	82	6
TF	45	4
Resistant		
MB	8	4
C172	3	4
RK	1	6

^a About 5×10^5 cells were infected with whole virus (multiplicity of infection, 300 PFU/cell) or with SV40 DNA I (multiplicity of infection, 0.02 PFU/cell) and then transferred to new petri dishes within 24 hr for the transformation assay. The number of transformed colonies is the total from 5×10^5 infected cells. The results are the average of two experiments.

DNA, on the other hand, required only approximately 10^4 infectious units per transformant in each of the cells tested.

Virus adsorption. The above results indicated that the resistance of certain human cell strains to transformation by whole virus was due to a block at an early step in infection (i.e., adsorption, penetration, or uncoating). Virus adsorption was studied by exposing representative susceptible and resistant fibroblast cultures to ³H-thymidine-labeled SV40. The cellular uptake of radioactivity was determined after incubation with labeled virus for 3 hr, whereas parallel cultures were assayed for T antigen-containing cells after 72 hr of incubation. Between 2 and 3% of the labeled virus became cell-bound during the adsorption period for each cell strain tested (Table 3). The fraction of T-antigen positives, nevertheless, was much higher in the susceptible strains (LS and 225) than in the two resistant strains (RM-12 and M436). These results suggest that virus adsorption is similar in transformation-susceptible and -resistant cells.

SV40 T-antigen induction with SV40 and adeno 7-SV40 hybrid virus. Adenovirus-SV40 hybrids are available which contain recombinant adeno-SV40 DNA enclosed in the adenovirus capsid (3). These viruses contain the genetic information necessary for the synthesis of both SV40 T antigen and adeno-T antigen. It was of interest to deter-

mine whether such a virus could carry the SV40 genome into resistant human cells in such a way as to remove the block to SV40 T-antigen synthesis. For this study, E46⁻, E46⁺, and SV40 viruses were used. Infection with E46⁻ resulted in a similar proportion of adeno-T antigen-containing cells at 48 hr for each cell strain tested; the percentage of positives was proportional to the amount of virus added. A virus inoculum which induced 1 to 5% Ad7-T-antigen positives was used. E46⁻ induced between 3.4 to 3.9% adeno-T antigen-positive cells in prototype susceptible, normal, and resistant strains (Table 4). With the hybrid virus (E46⁺), the percentage of adeno-T antigen-positive cells also was similar in each strain (3.2 to 3.8%) as was the percentage of SV40 T antigen-containing cells (3.9 to 4.7%). In marked contrast, SV40 itself induced more than 100 times as many T antigen-positive cells in strain LS as in the resistant culture RK (Table 4). Thus, induction of SV40 T antigen by the adeno-

TABLE 3. Infection of susceptible and resistant cell strains with ³H-uridine-SV40^a

Class	Cell strain	Input counts bound to cells	T-antigen positives
		%	%
Susceptible	LS	3.0	85.1
	225	2.8	83.0
Resistant	RM12	2.1	0.04
	M436	3.6	0.8

^a Infected with 1,000 PFU/cell. Virus adsorption was measured at 3 hr; T antigen was scored at 72 hr on cover slips from cultures exposed to the same virus inoculum.

TABLE 4. SV40 T-antigen induction by adenovirus-SV40 hybrid virus in susceptible, normal, and resistant human cell strains^a

Cell strain	T antigen-positive cells			
	SV40	Adenovirus 7 (E46 ⁻), adeno-T	Ad7-SV40 hybrid (E46 ⁺)	
			adeno-T	SV40 T
Susceptible LS....	12.8	3.9	3.4	4.1
Normal CM.....	3.0	3.4	3.1	3.9
Resistant RK.....	<0.1	3.7	3.2	4.7

^a Cultures containing approximately 5×10^5 cells were infected with SV40, E46⁻, or E46⁺ and transferred to new petri dishes with cover slips within 24 hr. Cover slips were stained for adeno-virus and SV40 T antigen at 48 hr.

virus hybrid was not affected by the block to T-antigen production seen with SV40.

Susceptibility of human cell strains to other viruses. The possibility that SV40 transformation-susceptible cells might also be more sensitive to infection by other viruses was examined. Two ribonucleic acid (RNA)-containing viruses (poliovirus type 1 and VSV) and one DNA-containing virus (herpes simplex) were titrated by plaque assay on several human strains. The susceptible group included cell strains from patients with Fanconi's anemia (LS, BL, AM). With poliovirus, titers ranged from 1.0 to 3.8×10^7 PFU/ml (Table 5). AGMK cells formed 10 to 50 times as many plaques as the human fibroblast strains. VSV and herpes simplex similarly showed no greater plaque-forming ability on SV40 transformation-susceptible cells than on representative cell strains from the other two classes (Table 5). These results indicate that the high sensitivity of certain human cell strains to infection by SV40 is specific for SV40 and not a result of a general increased susceptibility to all viruses.

DISCUSSION

Several reports described an increased susceptibility of cells from patients with Fanconi's anemia (11), Down's syndrome (12), and Kline-

felter's syndrome (D. Mukerjee, J. M. Bowen, D. E. Anderson, Cancer Res., *in press*) to transformation in vitro by SV40. These diseases are associated with chromosomal abnormalities and a high incidence of tumors (13). More recently, SV40 transformation-sensitive fibroblast strains were also detected among karyotypically normal cells derived from patients with tumors and their families (13). In an effort to determine the cause for the high susceptibility to transformation of such cells, the present studies were undertaken.

This report confirms earlier findings of marked differences in the levels of T-antigen production between human cell strains in response to infection by intact SV40 (1). The present studies demonstrate that isolated SV40 DNA is able to eliminate those differences. Similarly, much larger differences in T-antigen induction between such diverse species as the monkey, mouse, and rat kangaroo are removed through the use of SV40-DNA (2). Ad7-SV40 hybrid virus, which contains the genetic information for SV40 T-antigen induction within the Ad7 coat, also bypasses the block to T-antigen production in SV40-resistant cells. These two lines of evidence indicate that human cell sensitivity to T-antigen induction is largely influenced by the SV40 protein coat.

By using conditions described earlier for obtaining transformation of human fibroblasts with isolated SV40 DNA (2), it was possible to produce DNA-transformed colonies in many different human fibroblast strains. Although the levels of transformation differed markedly with whole virus, when the same cells were infected with isolated DNA, the differences were eliminated. From the experimental results, it is concluded that an event involving the virus coat is responsible for the differences in susceptibility of human fibroblast strains to transformation by intact SV40. The finding of a similar uptake of labeled virus by transformation-susceptible and -resistant cells suggests that the relative block to transformation in virus-resistant cell strains is at the level of virus penetration or uncoating, or both.

Potter et al. (9) have recently studied transformation and T antigen induction in human cells by SV40. They reported that for dividing cells, T antigen was observed in a higher percentage of transformation-sensitive cells from patients with Down's syndrome than in other strains. However, they noted that in confluent cultures, the reverse was true; T antigen was detected in a much higher proportion of resistant than of sensitive cells. Such a reversal in T-antigen levels in confluent cultures has not been observed in our laboratory. However, in the present study, dividing cultures were used to achieve the highest levels of trans-

TABLE 5. *Susceptibility of human strains to lytic viruses^a*

Cell strain	Virus titer ^b		
	Polio	VSV	Herpes simplex
Susceptible			
LS	2.0×10^7	2.8	5.0×10^7
BL	1.9×10^7	NT ^c	NT
FR	1.6×10^7	1.8×10^7	3.5×10^7
AM	2.5×10^7	NT	NT
AR	NT	3.2×10^7	2.6×10^7
Normal			
CM	1.0×10^7	3.2×10^7	7.0×10^7
BD	1.9×10^7	NT	NT
GS	NT	1.4×10^7	6.2×10^7
Resistant			
M413	3.0×10^7	2.0×10^7	10.0×10^7
M439	1.7×10^7	3.8×10^7	NT
C172	2.3×10^7	NT	NT
M447	3.8×10^7	1.9×10^7	8.2×10^7
Primary monkey kidney	50×10^7	50×10^7	NT

^a Cell monolayers were infected with serial 10-fold dilutions of the appropriate virus and overlaid with agar. Plaques were scored at 3 days for polio and vesicular stomatitis virus (VSV) and at 5 days for herpes simplex.

^b Expressed as plaque-forming units per milliliter.

^c Not tested.

formation and to minimize the possibility of virus spread. The differences in the effects of whole virus and viral DNA cannot be attributed to variations in cell density since all cultures were treated identically.

Potter et al. (9) also tested the sensitivity of transformation susceptible and resistant cell strains to other viruses including CELO, vaccinia, and influenza. They found that transformation-sensitive cells were not better able to support growth of these viruses. In this report, too, no correlation was found between the susceptibility of a cell strain to transformation by SV40 and its sensitivity to infection by either polio, VSV, or herpes simplex. These findings support the conclusion that cell strain variation in susceptibility to SV40 is specific for that virus. The interesting clinical correlation between tumor susceptibility and susceptibility to SV40 transformation continues to be investigated.

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