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Site of Host Restriction of Simian Virus 40 Mutants in an Established African Green Monkey Kidney Cell Line

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A previously described large-plaque morphology mutant of SV40 (SV-L) in primary African green monkey kidney (AGMK) cells has been shown to be restricted in the established AGMK line CV-1. The site of restriction is an early step in infection, involving the virus particle. A block in penetration or uncoating has been tentatively proposed. Restriction is observed in a number of other established lines, including monkey, human, and mouse. Independent large-plaque mutants were isolated in AGMK and their properties were compared with SV-L (temperature restriction, host restriction, and virion antigen). They can be separated into two classes. Both are restricted on CV-1, though to a lesser degree than SV-L. Furthermore, both are coat mutants as determined by immunological analysis of their virions, suggesting a relationship between coat mutants associated with large-plaque morphology in AGMK and host restriction in established cell lines. Temperature restriction is seen with one but not the other of the mutants.

The isolation and characterization of mutants of animal viruses would be expected to enhance our understanding of the interaction between virus and cell. Conditional lethal mutants (temperature or host range restricted) appear to be most useful, permitting the comparative study of the virus under both permissive and nonpermissive conditions.

The small oncogenic deoxyribonucleic acid (DNA) virus, simian virus 40 (SV40) replicates in several African green monkey kidney (AGMK) cell lines [CV-1 (9), VERO (6), and BSC-1 (7)] as well as AGMK primary cells. It might be possible, therefore, to isolate mutants restricted in their ability to infect normally susceptible cells of the same species. Previous study showed that wild-type SV40 stocks may be heterogenous in plaque morphology when plated on AGMK (14). Plaque assay of plaque-purified isolates on established cells revealed the large-plaque type (SV-L) to be restricted in established cell lines (15). The present study describes the nature of the restriction and relates it to other previously described properties of this mutant (plaque morphology, temperature restriction, and capsid antigen). Other independent isolates of large-plaque mutants were also compared. Host restriction has already been demonstrated as a useful genetic marker for SV40 rescued from transformed mouse cells (15).

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MATERIALS AND METHODS

Virus and cell lines. Primary AGMK and established cell line CV-1 were maintained and used for viral assay as previously reported (15). Isolation and growth of SV-L and small plaque (SV-S) mutants were in AGMK.

Virus purification. ^{32}P-labeled virus was prepared in AGMK cells as previously reported (11). Virus was purified by three centrifugations onto 40% KBr. The mixed complete and incomplete virions were then centrifuged into 34% sucrose through 10% sucrose in 0.01 M NaCl, 0.003 M $MgCl_2$, 0.01 M tris(hydroxymethyl)aminomethane-hydrochloride, *p*H 7.4 (2), at 23,000 rev/min for 90 min at 4 C in an SW 25.1 rotor. One peak containing virus was observed in the middle of the tube. A second peak of radioactivity remained at the top of the tube and was designated "disrupted virus." Ninety per cent of the radioactivity of the viral fraction and $<10\%$ of the "disrupted virus" fraction was sedimentable at 35,000 rev/min in 60 min.

Unlabeled virus, prepared in AGMK cells, was purified by centrifugation onto 40% KBr and equilibrium centrifugation in CsCl (ρ 1.34) twice.

Infectivity of viral DNA. DNA was extracted from CsCl-purified virus of each plaque type by the method

of Berns and Thomas (1). Infection of either AGMK or CV-1 cells was as reported by McCutchen and Pagano (10) using 0.1 mg of diethylaminoethyl (DEAE)-dextran (Pharmacia, 2 × 10^6 molecular weight) in 0.2 ml per plate.

The DNA preparations were standardized on the basis of plaque-forming units (PFU) on AGMK.

Virus adsorption. Purified ^{32}P-labeled virus was diluted in Eagle medium with 1% fetal calf serum and 0.2 ml (500 counts/min) was incubated with confluent monolayers of AGMK (4 × 10^6 cells) or CV-1 (1.6 × 10^6 cells) in 60-mm petri dishes, for varying periods of time at 37 C. The inoculum was removed, and the monolayers were washed three times with a total volume of 5 ml of phosphate-buffered saline (PBS), *p*H 7.4. All washes were pooled and precipitated at a final concentration of 5% trichloroacetic acid in the cold for determination of "unabsorbed" counts. The monolayers were scraped into 5 ml of PBS and similarly precipitated for cell-bound ("adsorbed") counts. Fractions were counted on filters (Millipore Corp., Bedford, Mass) in PBD (Nuclear-Chicago; phenylbiphenyloxadiazole-1,3,4 in toluene) by liquid scintillation spectroscopy. In controls in which virus was incubated on petri dishes in the absence of cells, 90 to 95% of the counts were recovered as unabsorbed with 1 to 2 of the input counts in the final wash.

Serological assays. The microcomplement fixation test was performed by the method of Sever (13) with two units of complement. Tumor antigen (T antigen) was assayed by using a hamster antiserum against a virus-free, SV40-induced, transplantable tumor, and viral antigen (V antigen) was assayed by anti-SV40 hamster serum prepared with purified SV40.

Complement fixation antigen production was measured by infecting confluent monolayers in 60-mm petri dishes. At various times after infection, the medium was decanted and the cells were harvested in 1 ml of PBS. The suspension was frozen and thawed and assayed directly. Titers given are the reciprocal of two-fold dilutions.

The radioisotope precipitation and inhibition techniques as well as the reagents used were previously described (11).

RESULTS

Growth of SV40 in CV-1 cells. Takemoto et al. (14) reported that SV40 of different plaque morphology can be isolated on AGMK. These have been designated SV-L for large-plaque and SV-S for small-plaque morphology. These isolates are genetically stable after multiple plaque purifications and preparations of virus stocks. However, when these viruses are plaqued on CV-1 cells, SV-L but not SV-S is markedly restricted. The efficiency of plaque formation for SV-L on CV-1 is 0.1 to 1% of that on AGMK, whereas that of SV-S is 10 to 100%. Furthermore, the size of the SV-L plaques is reduced, often pinpoint, even at 21 days (15).

Growth curves of these viruses in liquid media also show a restriction of SV-L (Fig. 1).

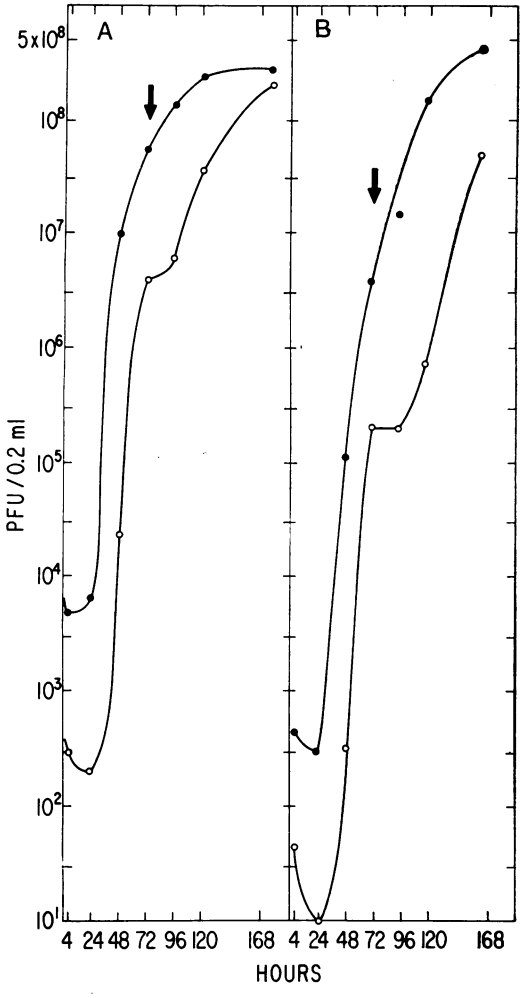

FIG. 1. *Growth curve of SV-L and SV-S in CV-1 cells. CV-1 monolayers were infected at a multiplicity of infection of 1 PFU per cell (A) or 0.01 PFU per cell (B) with SV-L (○) and SV-S (●) and harvested at the time indicated postinfection in 5 ml. Virus yield and input were determined by PFU on AGMK. Arrow designates the break in the growth curve (72 hr postinfection).*

CV-1 monolayers were infected with either SV-S or SV-L at multiplicities of infection of 1 and 0.01 per cell (based on PFU of each virus on AGMK). After 2 hr of incubation for adsorption of virus, the inoculum was removed; the monolayers were washed and reincubated with 0.5 ml of a rabbit anti-SV40 antiserum to neutralize residual unadsorbed virus. After 2 hr, the antiserum was removed, the monolayers were washed with medium and 5 ml of fresh medium was added. At various times thereafter, the cells and medium were har-

vested together and assayed for PFU with AGMK cells.

The growth curves suggested a block in the initial stages of infection for SV-L in CV-1. The results were compatible with either a reduced efficiency of adsorption or penetration (i.e., resistance to antibody neutralization). Both viruses showed a period of rapid production at 36 to 72 hr. At 72 to 96 hr (approximately the time of completion of a single cycle by most of the virus), SV-L but not SV-S reached a temporary plateau, compatible with a reduced efficiency of SV-L to initiate a second cycle. Ultimately, however, high yields of SV-L are attained, though very much delayed, and, even at this time, only marginal cytopathic effect is observed.

Virus antigen induction. SV40-induced antigens can be used to delineate the infectious cycle of the virus. T antigen is an early protein, prior to viral DNA replication, whereas V antigen follows viral DNA replication (12). When both viruses were assayed on monolayers of each cell type, it was found that T and V antigen induction was depressed for SV-L in CV-1 when compared to levels obtained either with SV-S in CV-1 or SV-L in AGMK. Complement fixation data are summarized in Table 1. The finding of reduction of both early and late antigen suggests that an early block is responsible, prior to or at the level of T antigen. The finding of V antigen in the absence of T antigen in CV-1 at low multiplicity probably reflects the difference in sensitivity of the complement-fixation assays. Fluorescent antibody studies confirmed a reduced efficiency of SV-L in inducing T and V antigens in CV-1 (decreased percentage of positive cells).

Infection with viral DNA. The early stages of viral infection may be broadly divided into two categories: those related to the interaction of the viral particles with the cell (adsorption, uncoating, etc.) and those related to the subsequent reading of the viral genetic information (transcription, translation, etc.). One might postulate, therefore, that studies with infectious DNA could be used to determine which of these two early stages were blocked for SV-L in CV-1 cells. Analysis of a first-cycle yield experiment was performed to eliminate the complication of restriction of progeny virus infection.

Figure 2 shows the control experiment in which equivalent multiplicities of infection of SV-L virus and DNA were compared in permissive AGMK cells; no difference was observed. When a multiplicity of less than one per cell was used, it was found that there was a reproducible relationship between the virus yield and the input (in PFU). Subsequent data are presented in that form, permitting comparison between different experiments. Table 2 shows the results for virus and viral DNA in the two systems. SV-S DNA and virus are equally effective in inducing progeny virus in CV-1 cells, as with SV-L in AGMK. However, SV-L DNA overcomes the restriction in CV-1 cells, i.e., approximately 100 times more virus is induced with infectious DNA than when a comparable amount of virus is used for infection. Indeed, the yield of virus during the first cycle (36, 48 hr) approaches that of SV-L (DNA or virus) in the permissive AGMK cells.

Virus adsorption. Inasmuch as an early step appeared to be the site of restriction, adsorption of radioactively labeled purified virus (^{32}P) was investigated in the two systems. The final preparations of SV-L and SV-S were comparable in specific activity (5,000 counts per min per complement-fixing unit) and deoxyribonuclease resistance (90%).

Figure 3 shows the adsorption kinetics in the

TABLE 1. *Induction of SV40 antigens*[a]

Virus	MOI[b]	Cell line	T antigen (hr postinfection)			V antigen (hr postinfection)		
			24	48	72	24	48	72
SV-S	15	AGMK	NT[c]	8	NT	NT	32	NT
	40	CV-1	1	8	NT	0	64	NT
	1.5	AGMK	1	4	8	0	8	12
	4	CV-1	0	0	2	0	2	8
SV-L	25	AGMK	NT	16	NT	NT	32	NT
	80	CV-1	0	2	NT	0	4	NT
	1.5	AGMK	0	4	8	0	16	24
	4	CV-1	0	0	0	0	0	2

[a] Induction of complement-fixing antigen was determined in parallel in CV-1 and AGMK monolayer cultures as described.

[b] Multiplicity of infection.

[c] Not tested.

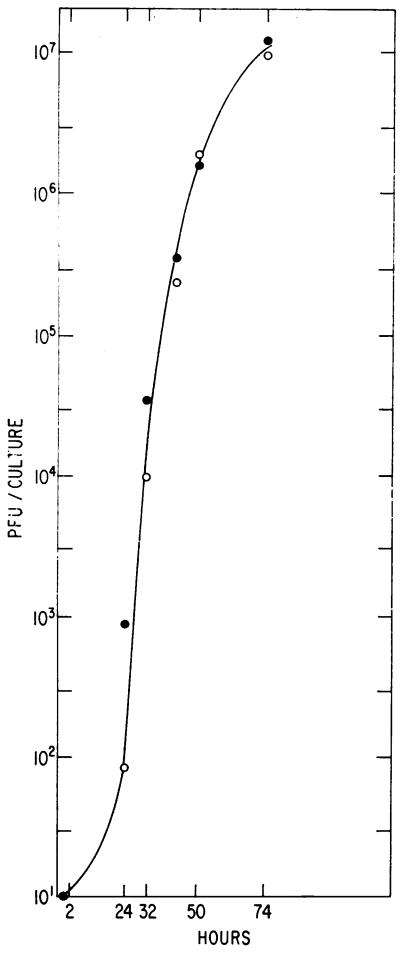

FIG. 2. *Virus induction with virus or viral DNA in AGMK. AGMK monolayers were infected at a multiplicity of infection of 0.01 PFU per cell with either virus* (○) *or viral DNA* (●). *See Table 2 for conditions. Virus yield and input were determined by PFU on AGMK.*

two systems. All experiments were performed with the same preparations at an estimated multiplicity of infection of one to five per cell. Part A shows the trichloroacetic acid precipitable counts which were cell-associated after repeated washing. On AGMK cells, both viruses were rapidly adsorbed and reached a plateau within 60 min at 50 to 60% of input counts. Adsorption of both viruses was significantly reduced on CV-1 cells, but the difference between SV-L and SV-S was less than twofold, insufficient to explain the much greater difference in infectivity and plaquing efficiency. The decreased adsorption on CV-1 as compared to AGMK may be due to the lower cell numbers in the monolayers. Essentially similar results were obtained (Fig. 3B) when "unadsorbed" counts were determined (on CV-1 cells only).

In view of the low level of adsorption observed with both SV-L and SV-S on CV-1, we cannot be certain that a significant number of counts "adsorbed" actually represent DNA released from the particles during the incubation. This possibility is made less likely when adsorption of "disrupted virus" is studied (Fig. 3B). The material used was the fraction which remained at the top of the sucrose gradient in the final step of virus purification, presumably free DNA or DNA in association with protein. In this case, adsorption was very rapid, as shown by loss of counts from medium. Cell-bound counts are not shown in Fig. 3; they reach a peak by 60 min (10 to 20% of input counts) with a decrease at 120 min. Both of these findings are different from the data obtained for intact virus.

It would thus appear that, on the basis of the growth curve and antigen induction, an early step in virus infection is responsible for the restriction of SV-L on CV-1. This block involves the viral particles (inferred by its being overcome by viral DNA) at a step subsequent to the binding of viral particles, perhaps inefficient penetration or uncoating.

Relation of host restriction to other SV-L properties. Previous studies from this laboratory have demonstrated additional differences between SV-L and SV-S: (i) temperature restriction of the growth of SV-L but not SV-S in AGMK (14) and (ii) immunological differences in their virions (11). It is therefore possible that these properties, including host restriction and plaque morphology, are all reflections of capsid differences, possibly of a single type. Since SV-L and SV-S were both isolated from a multiple-passaged wild-type stock, however, it is not possible to determine easily the relationship of the two viruses.

One approach would be to isolate other large-plaque variants from SV-S and determine which, if any, of the above properties are related. Ten large plaques were picked from AGMK plates infected with 10^3 to 10^4 PFU of SV-S and triply plaque-purified on AGMK. Two classes of mutants were obtained when characterized for each of the above properties. SV-L and SV-S are included for comparison. Temperature restriction was determined in AGMK by comparing complement-fixing antigen induction and virus replication at 37 and 40 C (Table 3). Mutant S/612 was temperature-sensitive as SV-L, but S/531 was not. Host restriction was determined by efficiency of plaque formation and complement-fixing antigen production in CV-1 and AGMK. Both mutants have reduced efficiency of formation of plaques in CV-1, as SV-L (Table 4). The size of the plaques

TABLE 2. *Comparative infectivity of virus or viral DNA*[a]

Virus or DNA (MOI)[b]	AGMK (hr postinfection)			CV-1 (hr postinfection)		
	36	48	72	36	48	72
SV-S DNA (0.0006–0.001)				0.06[c]	21	
SV-S Virus (0.0003–0.06)				0.03	18	760
Ratio of DNA/Virus				2	1.3	
SV-L DNA (0.001–0.03)	1.8[c]	80	600	0.83[c]	55	
SV-L Virus (0.008–0.8)	2	400	1,900	0.006	0.55	45
Ratio of DNA/virus	0.9	0.2	0.3	140	100	

[a] Confluent monolayers were infected with virus or viral DNA (in 0.1 mg of diethylaminoethyl-dextran) in 0.2 ml. After adsorption (2 hr for virus and 15 min for DNA), the monolayers were washed, and 5 ml of Eagle medium with 1% fetal calf serum was added. Cultures were harvested at 36, 48, and 72 hr postinfection. Virus yield per culture was determined by plaque assay on AGMK.

[b] Multiplicity of infection per cell was calculated on the basis of PFU in AGMK.

[c] Ratio of virus yield per culture divided by input of PFU.

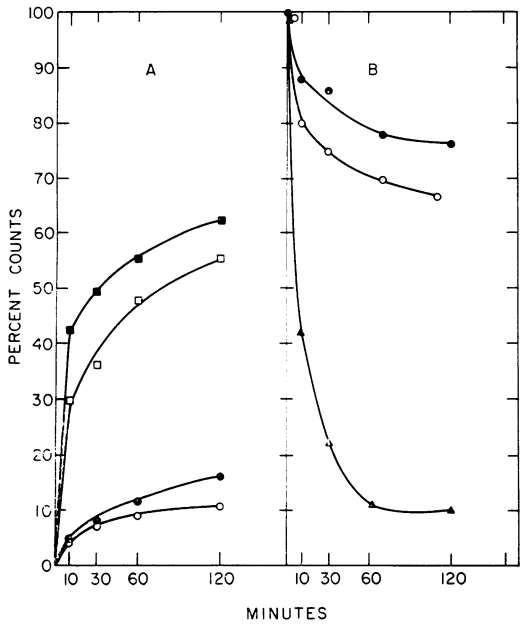

FIG. 3. *Adsorption of radioactively labeled virus to AGMK and CV-1. CV-1 and AGMK monolayers were incubated with radioactive virus preparations as described. SV-L (○) on CV-1, SV-L (□) on AGMK, SV-S (●) on CV-1, SV-S (■) on AGMK, or "disrupted virus" (▲). (A) Cell-bound counts ("adsorbed"); (B) unbound counts ("unadsorbed").*

formed is also reduced. Antigen induction, however, is not as markedly reduced in CV-1 for S/612 and S/531 as for SV-L at high multiplicities of infection (as in Table 5), suggesting an intermediary degree of restriction (i.e., reflected only on multiple-cycle growth). A block at a step different from SV-L has not been ruled out, however.

TABLE 3. *Temperature restriction in AGMK*[a]

Virus	37 C			40 C		
	T[b]	V[b]	PFU[c]	T[b]	V[b]	PFU[c]
SV-S	8	8	4×10^8	8	4	3×10^7
SV-L	>16	16	5×10^8	>8	32	5×10^4
S/612	>16	32	1×10^9	>8	32	2×10^5
S/531	>16	32	2×10^8	>8	16	1×10^8

[a] Complement-fixing antigen and virus yield (separate experiments) were determined on replicate cultures maintained at 37 and 40 C.

[b] Complement-fixing antigen induction was determined at 48 hr postinfection as described. The multiplicities of infection for the different virus stocks were as follows: SV-S, 1; SV-L, 100; S/612, 100; and S/531, 100.

[c] Virus yield per culture at day 6 postinfection (moderate cytopathic effect observed at both temperatures) was determined by plaque assay on AGMK at 37 C. The multiplicities of infection for the different virus stocks were as follows: SV-S, 1; SV-L, 1; S/612, 6; and S/531, 1.

The immunochemical properties of the virions of the mutants were compared by using inhibition of the radioisotope-labeled virus precipitation test. Presence of an antigen is demonstrated by complete inhibition (>90%) of precipitation. As previously reported (11), SV-L contains an antigen not present in SV-S, which can be detected by comparative inhibition of the precipitation of radioactively labeled SV-L by antiserum prepared against SV-L. S/612 contains this SV-L antigen but S/531 does not (Fig. 4).

Antigens common to both SV-L and SV-S can also be detected by inhibition in the radioisotope precipitation test as previously described (11). In this case, antiserum prepared against SV-S and

TABLE 4. *Host restriction: efficiency of plaque formation*[a]

Virus	PFU in cell line:	
	AGMK	CV-1
SV-S	2.4×10^6	7×10^5
SV-L	2.6×10^6	1.4×10^4
S/612	3.3×10^6	3.4×10^4
S/531	3.2×10^6	1.8×10^4

[a] Comparison of plaque-forming units per 0.2 ml on monolayers.

TABLE 5. *Host restriction: antigen induction*[a]

Virus	MOI[b]	AGMK		CV-1	
		T	V	T	V
SV-L	50–100	16	32	2	4
S/612	50–100	32	64	16	32
S/531	50–100	16	64	8	32

[a] Complement-fixing antigen induction was determined at 48 hr postinfection in parallel cultures.
[b] Multiplicities of infection.

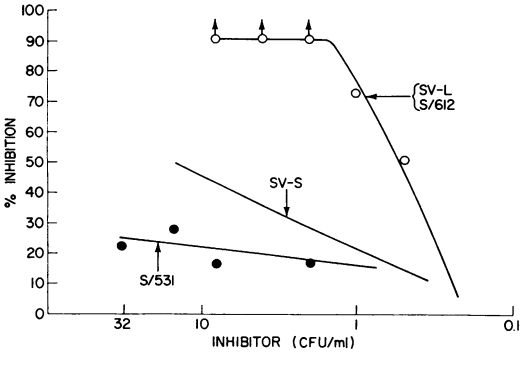

FIG. 4. *Radioisotope precipitation (RIP) inhibition of SV-L specific antigen(s). RIP reagents: SV-L (^{3}H-thymidine, 0.6 complement fixation units/ml), monkey anti-SV-L (diluted 1:10,000), and rabbit anti-human gamma-globulin (diluted 1:20); control precipitation, 75%. Virus inhibitors: S/531 (●) and S/612 (○). Inhibition curves obtained with SV-S and SV-L under the same conditions in multiple experiments are drawn as designated. The SV-L curve was superimposable on that of S/612.*

radioactively labeled SV-L are used (Fig. 5). Both SV-S and SV-L completely inhibit, as expected. S/612 also inhibits completely. S/531, on the other hand, only partially inhibits, indicating that it is missing at least one antigen present in SV-S (i.e., that shared between SV-S and SV-L). These characteristics are summarized in Table 6.

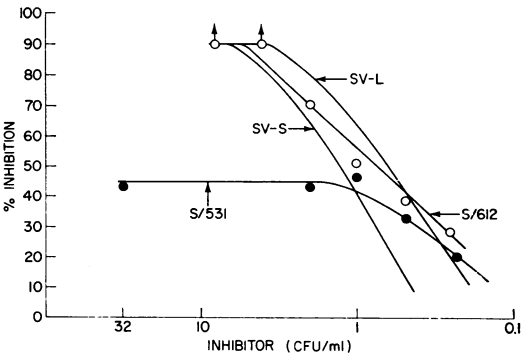

FIG. 5. *Radioisotope precipitation (RIP) inhibition of SV-L and SV-S common antigen(s). RIP reagents: SV-L (^{3}H-thymidine, 0.6 compliment fixation units/ml), monkey anti-SV-S (diluted 1:7,500) and rabbit anti-human gamma-globulin (diluted 1:20); control precipitation, 65%. Virus inhibitors: S/531 (●) and S/612 (○) with standard curves for SV-S and SV-L as in Fig. 4.*

TABLE 6. *Properties of SV40 plaque mutants*

Virus	Plaque morphology (AGMK)	Temp restriction[a] (AGMK)	Host restriction[b] (CV-1)	Capsid antigen[c]	
				SV-L	Common
SV-S	Small	TS+	CV+	−	+
SV-L	Large	TS−	CV−	+	+
S/612	Large	TS−	CV±	+	+
S/531	Large	TS+	CV±	−	−

[a] (+) Less than 10 times reduction in virus yield at 40 C versus 37 C; (−) greater than 1,000 times reduction in virus yield at 40 C versus 37 C.
[b] (+) Less than 10 times reduction in PFU and less than two to four times reduction in antigen induction in CV-1 versus AGMK; (−) 100 times reduction in PFU and greater than 8 times reduction in antigen induction; (±) = 100× reduction in PFU and less than two to four times reduction in antigen induction.
[c] (+) Complete inhibition of RIP precipitation; (−) = partial inhibition of radioisotope precipitation at very high inhibitor-to-labeled virus ratio (20:1). See reference 11 for more detailed discussion.

S/612 and SV-L appear to be very closely related, as indicated by their similar properties of temperature restriction and virion antigens. S/612 can be distinguished from SV-L by the moderate degree of host restriction of S/612. The class of mutants represented by S/531 is quite distinct from either of the other classes of SV40. Though it has large-plaque morphology on AGMK and moderate host restriction on CV-1, its virion is different, as seen in its loss of an antigenic deter-

minant (and the presumable gain of a new antigen) and its lack of temperature restriction at 40 C.

In conclusion, two points can be made concerning the interrelationship of the different large-plaque mutants and SV-S. First, a probably single event may result in multiple biochemical and biological differences: virion antigens, temperature restriction, and host restriction, as seen for S/612. The similarity of properties for S/612 and SV-L suggests that the latter is also derived from SV-S. Secondly, not all of these properties need occur together, as seen for S/531.

DISCUSSION

SV40 is routinely propagated in various AGMK cell lines in different laboratories. We have shown in this study that the growth properties of at least some isolates of SV40 may differ markedly among such cell lines (plaque morphology and virus yield). Analysis of the nature of the restriction of SV-L in CV-1 indicates that a block in an early event in infection, involving the viral particle but not the viral DNA, is responsible. Studies with radioactively labeled virus suggest that this block is located at a stage subsequent to adsorption. Preliminary data indicate that there is no difference in release of SV-L or SV-S in either cell line [in contrast to that suggested for plaque morphology mutants of polyoma (3)], and that progeny SV-L propagated in CV-1 are similarly restricted. Though the molecular basis for restriction remains undefined, we would tentatively propose that penetration or uncoating of the viral particle is affected.

All the mutants in this study, which were selected for their large-plaque morphology in AGMK (SV-L, S/612, S/531), share the property of CV-1 restriction, though to varying degrees. Furthermore, restriction occurs in the other established AGMK lines of VERO and BSC-1 as well (Ozer and Takemoto, *unpublished data*), although the mechanism of restriction has not been explored. Todaro and Takemoto (Proc. Nat. Acad. Sci. U.S.A., *in press*) have also found that SV-L is inefficient in inducing T antigen and transformation in mouse and human cell lines. Similarly, S/612 and S/531 have reduced efficiency in inducing T antigen and transformation in these cells, to a degree intermediary between SV-S and SV-L (analogous to that seen in CV-1; Ozer, Takemoto, and Todaro, *unpublished data*). Consequently, selection of mutants capable of forming large plaques in AGMK correlated with a marked inability to infect established cell lines from a variety of species.

These mutants also share the property of being coat mutants of SV40, having virions immunologically distinct from SV-S. The selection of large-plaque morphology mutants on AGMK, therefore, provides a simple technique for obtaining coat mutants. In two cases (SV-L, S/612), they share an antigenic change to a new specificity, whereas in the case of the third (S/531), the loss of a prior specificity has been demonstrated. The antigenic character of SV-S itself is stable on plaque purification. The chemical basis for the antigenic changes is unknown. Comparison of the amino acid compositions of SV-L and SV-S reveal only minor differences (Ozer and Erickson, *unpublished data*). More definitive analysis by comparison of the tryptic peptides of the virions is now in progress. One might expect the chemical differences among the various mutants to be slight, especially S/612 and S/531, which were derived from recently plaque-purified SV-S. In view of the demonstration in this paper that CV-1 restriction involves the virion of SV-L (and probably that of S/612 and S/531 as well), it is likely that the antigenic changes are related directly to the host restriction. Vogt and Ishizaki (17) also observed the relationship between virion structural differences (immunological) and host range with Rous sarcoma virus and chicken cells (8, 17). The nature of the immunological differences appeared marked, almost all serological cross-reaction being lost. In the case of the SV40 mutants, however, the change is subtle, not being detectable by complement fixation, for example (11).

These results, indicating that slight chemical modification of the virion can be reflected in marked host range restriction, may have significance in the studies involving "rescue" of SV40 from transformed cells by fusion technique. Several workers (5, 15) have shown that SV40 can be rescued from all mouse cells transformed by virus, and Takemoto et al. (15) have furthermore shown that the virus retains its genetic markers. When mutagenized virus is used in transforming mouse cells, as by Dubbs and Kit (4) using ultraviolet-irradiated virus stocks, virus is not recoverable in some instances. In the latter case, however, only a single monkey cell line was employed (CV-1), and one may be dealing with mutants of altered host range which could be detected by using AGMK for recovery attempts. Indeed, the phenomenon of altered host range has been well-demonstrated to be operative in Rous-transformed avian cells previously thought to be non-producers (16).

Finally, plaque mutants of the type described may be useful for genetic studies with SV40. It is unknown whether the various properties of temperature restriction, host restriction, and

virion antigens represent changes in a single cistron or multiple cistrons. Complementation and recombination studies with these mutants should answer this question.

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