VARIATION IN THE ONCOGENIC POTENTIAL OF HUMAN ADENOVIRUSES CARRYING A DEFECTIVE SV40 GENOME (PARA)*

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The biological properties of a human adenovirus that has incorporated defective SV40 genetic material within its adenocapsid have been extensively studied (1, 2). The particle containing the defective SV40 genome is known as PARA (2). Its presence confers new properties on the adenovirus population which now replicates in simian cells (3), carries the genetic information for the synthesis of SV40 tumor (T) antigen (4, 5) and SV40 specific transplantation antigens (6), and is oncogenic in newborn hamsters (1, 7).

Genetic material from PARA can be transferred to other human adenoviruses by a process called transcapsidation (8, 9). As a result of such transfer to type 2, this previously nononcogenic adenovirus produced tumors when injected into newborn hamsters (7, 10). These tumors were characteristic of either SV40-induced tumors, adenovirus-like tumors, or contained elements of both types. Hamster cells transformed in vitro by PARA–adenovirus 2 yielded similar tumors when inoculated into weanling hamsters (11).

The PARA component has also been transferred to seven other human adenovirus serotypes. The in vitro properties of these newly created populations have been reported (12). To determine whether the acquisition of defective SV40 conferred oncogenic potential to all adenoviruses, these populations were tested by injecting them into newborn hamsters. The ability of these viruses to protect hamsters against challenge with cells transformed by SV40 virus was also investigated. The results of these studies, which indicate that the acquisition of the SV40 genome does not necessarily confer oncogenic potential to an adenovirus, are presented in this paper.

Materials and Methods

Viruses.—The history of the PARA-adenovirus type 7 (stock SP2) used in this study has been described in detail (6). The virus was passed two additional times in primary African

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green monkey kidney (GMK) cells. Adenovirus type 2 was a local strain. Adenovirus types 1, 5, 6, 14, and 16 were the prototype virus strains obtained from the National Communicable Disease Center, Atlanta, Ga. Adenovirus type 21 was obtained from the Amercian Type Culture Collection, Rockville, Md. These adenoviruses were passed twice in KB cells and once in primary human embryonic kidney (HEK) cells in our laboratory. The HEK cells were supplied by the Human Tissue Procurement Program, National Cancer Institute, Bethesda, Md. Adenovirus type 3 had been obtained from Wyeth Laboratories (Philadelphia, Pa.) and had been passed four times in KB cells, plaque-purified twice in HEK cells, and then passed once in the same cells. None of these viruses carried genetic information needed to induce known SV40 antigens and none could replicate well in simian cells in the absence of SV40 or PARA (12).

The process of transferring the PARA component to these human adenovirus populations has been described (8, 12). Terminal dilutions of PARA–adenovirus 7 were plated onto GMK cells growing in 60 × 15 mm plastic Petri dishes. The cells were simultaneously inoculated with a saturating amount of the human adenovirus which alone caused no plaques in the simian cells. The plaques that were produced in doubly infected cells were picked and tested for transcapsidants. Residual adenovirus type 7 was neutralized by exposing the virus from the plaque to type-specific adenovirus 7 antiserum for 1 hr at 37°C. Stocks were then prepared by inoculating the virus onto GMK cells growing as monolayers in 16 oz prescription bottles. The cultures were harvested when cytopathic effects were maximal by two cycles of quick-freezing in an alcohol-dry ice bath followed by thawing at 37°C. The cell debris was removed by low speed centrifugation and the supernatant used as virus stock.

SV40 was the Baylor reference strain used in previous studies reported from this laboratory (13, 14).

Virus Assays.—Since PARA–adenovirus populations are made up of two kinds of particles, a true adenovirion and an adenovirus capsid containing defective SV40 genetic material (PARA), titrations for each component were carried out separately. The human adenovirus component was titrated in HEK cells by the plaque technique (15). The HEK cells were grown in 35 × 10 mm plastic Petri dishes in Melnick's lactalbumin hydrolysate medium (M-E) with 10% fetal calf serum and 0.23% $NaHCO_3$. All media contained 100 units of penicillin and 100 μg of streptomycin per milliliter. 10-fold dilutions of the virus were plated in 0.1 ml amounts onto replicate cultures, and the virus was allowed to adsorb for 1 hr at 37°C with frequent manual rotation of the plates. Then, 1.5 ml of overlay containing 1% agar, 10% fetal calf serum, and 0.23% $NaHCO_3$ in Eagle's medium was added. A second overlay with neutral red was added 1 wk later, and plaques were counted on the 10th day after inoculation.

For titration of PARA, GMK cells were grown in 60 × 15 mm plastic Petri dishes in Melnick's lactalbumin hydrolysate (M-H) with 2% calf serum and 0.08% $NaHCO_3$. 10-fold dilutions in 0.1 ml amounts were plated onto the GMK cells in the presence of a saturating amount of nonreplicating helper human adenovirus (16). After adsorption of the virus for 1 hr at 37°C, 5 ml of an overlay similar to the one described above were added. A second overlay with neutral red was added 1 wk later; the plaques were counted 10–12 days after inoculation of the virus.

Immunofluorescence Techniques.—Cells were grown on 15 mm round cover slips. The cells were washed three times with warm Tris buffer (pH 7.4), air dried, and fixed in acetone for 3 min. They then were reacted with antisera for 30 min, washed 3 times with Tris buffer, and reacted with fluorescein-labeled antiglobulin for 30 min. After three washings with Tris buffer followed by drying at room temperature, the cover slips were mounted in Elvanol and examined with a Zeiss fluorescence microscope. The details of the preparations of the reagents and the procedures employed have been described (14, 17).

Sera from hamsters with tumors were tested for the presence of SV40 T antibodies by react-

ing the serum with GMK cells inoculated 24 hr previously with SV40. Fluorescein-labeled anti-hamster globulin was then added. The same sera were tested for the presence of antibodies against adenovirus T antigen by reacting the serum with HEK cells inoculated 24 hr earlier with the adenoviruses. Tumor cells were tested for the presence of SV40 T antigen by reacting the cells with sera of hamsters bearing tumors induced by the inoculation of SV40-transformed, but virus-free (18), hamster cells (H-50 cell line). The cells were tested for SV40 virus (V) antigen by reacting them with fluorescein-labeled anti-SV40 monkey serum. Adenovirus T antigen was detected by reacting tumor cells with serum from hamsters bearing tumors induced by adenovirus type 12. The presence of adenovirus V antigen in the tumor cells was detected with type-specific anti-adenovirus 7 rabbit serum.

Animal Experiments.—Newborn hamsters were inoculated subcutaneously within 24 hr of birth with 0.1 ml of medium containing a PARA-adenovirus or a human adenovirus. The animals were weaned 3 wk after birth and checked weekly for tumor development. When the tumors measured greater than 10 × 10 mm, the animals were bled out and the sera collected.

Some of the tumors were removed, trypsinized, and the cells grown in 16 oz prescription bottles in Eagle's medium with 10% fetal calf serum. The cells were tested for the presence of virus-specific antigens by immunofluorescence techniques. The tumor cells were also plated onto GMK and HEK cells in attempts to isolate virus. One blind passage of cell cultures not showing cytopathic effects was carried out. Tumor cell extracts (20%), prepared by two alternate cycles of quick-freezing cells from the tumor in an alcohol-dry ice bath followed by thawing at 37°C, were also examined for the presence of virus particles with the electron microscope.

Transplantation rejection experiments were carried out by inoculating weanling hamsters three times at weekly intervals with various viruses followed by challenge with cells transformed by SV40 (H-50 cell line). The dose of the immunizing viruses was adjusted so that each hamster was inoculated with the same amount of adenovirus in the corresponding PARA-adenovirus population. Even though the amount of the adenovirus was therefore constant for each serotype, these dilutions resulted in differing inocula of PARA serotypes per animal. The actual titers of both PARA and adenovirus inoculated are given in Table V.

The H-50 cells, derived originally from a hamster tumor induced by SV40 (19), had been passed about 100 times in tissue culture and were then transplanted serially two times in hamsters to increase their transplantability. They were reestablished in tissue culture and subsequently passed 10 times. The cells are free of infectious SV40 (18) but contain SV40 intranuclear T antigen (20) and surface (S) antigen demonstrable by immunofluorescence (21). The challenge H-50 cells were inoculated subcutaneously using 10-fold dilutions ranging from 100 to 100,000 cells per animal. Eight animals were used for each concentration of cells; i.e., 32 animals were employed for each virus. The number of cells required to produce tumors in 50% of the recipient animals (TPD_{50}) in each group was calculated by the method of Reed and Muench.

RESULTS

Oncogenicity of PARA-Adenovirus Populations.—The various PARA-adenovirus populations and the parent serotypes were injected subcutaneously into groups of new born hamsters within 24 hr after birth. The transfer of PARA to adenovirus types 3, 14, 16, and 21 failed to enhance the oncogenic potential of these viruses (Table I). Only one animal in this group (inoculated with PARA-adenovirus 3) developed a tumor within the 9 months observation period. None of the animals inoculated with the parent serotypes (i.e., before

the transfer of the PARA genome) developed a detectable neoplasm during this time. This is in contrast to animals inoculated with the original PARA-adenovirus 7 which induced tumors in 49% of the animals inoculated (Table I). Some of the groups were inoculated in conjunction with experiments reported here, but the large number of animals cited in Table I represent accumulated totals, some of which have been used in a previous report (7). These viruses

TABLE I

Oncogenicity of Human Adenoviruses with DNA of Intermediate GC Content Before and After the Acquisition of Defective SV40 (PARA)

Virus inoculated	Plaque-forming units inoculated		Hamster results				
	PARA*	Adenovirus‡	No. inoculated	No. weaned	No. with tumors	Per cent with tumors§	Latent period
							wk
Adenovirus 3	—	$10^{6.0}$	50	12	0	0	—
PARA-adeno 3	$10^{3.1}$	$10^{6.0}$	78	20	1	5	19
Adenovirus 7	—	$10^{5.0}$–$10^{7.0}$	248	117	0	0	—
PARA-adeno 7‖	$10^{5.0}$	$10^{5.7}$–$10^{6.6}$	390	197	97	49	17–27
Adenovirus 14	—	$10^{6.2}$	95	12	0	0	—
PARA-adeno 14	$10^{3.0}$	$10^{5.5}$	90	10	0	0	—
Adenovirus 16	—	$10^{6.5}$	85	9	0	0	—
PARA-adeno 16	$10^{4.3}$	$10^{6.4}$	84	14	0	0	—
Adenovirus 21	—	$10^{5.1}$	78	20	0	0	—
PARA-adeno 21	$10^{3.3}$	$10^{5.2}$	79	19	0	0	—

* Obtained by plaque assay in green monkey kidney cells coinfected with a helper adenovirus.

‡ Obtained by plaque assay in human embryonic kidney cells.

§ Based on number weaned.

‖ From reference 7.

are all members of hemagglutinating group I (22) with demonstrated oncogenesis (23) and an intermediate (50–53%) composition of guanine plus cytosine (GC) in the viral DNA (24).

Similarly, newborn animals were inoculated with PARA-adenovirus or the parent serotypes belonging to hemagglutination group 2 (22). These viruses, including adenovirus types 1, 2, 5, and 6, have thus far been nononcogenic in newborn hamsters and contain DNA with a high (56–58%) GC content (24). None of the parent serotypes produced tumors in the hamsters within the observation period (Table II). However, all viruses of this group carrying PARA

became oncogenic. PARA–adenovirus 1 induced tumors in 35% of the animals weaned; it took from 22 to 29 wk for these tumors to become palpable. A similar percentage of animals inoculated with PARA–adenovirus 6 developed tumors. PARA–adenovirus 2 induced tumors in 19% of the animals weaned. Although only 3 of 51 animals (6%) developed tumors after inoculation of PARA–adenovirus 5, these tumors all developed very rapidly with a latent period of only 8–10 wk.

Virologic and Serologic Analysis of Tumors and Hamster Sera.—It had been

TABLE II

Oncogenicity of Human Adenoviruses with DNA of High GC Content Before and After the Acquisition of Defective S40 (PARA)

Virus inoculated	Plaque-forming units inoculated		Hamster results				
	PARA*	Adenovirus‡	No. inoculated	No. weaned	No. with tumors	Per cent with tumors§	Latent period
							wk
Adenovirus 1	—	$10^{6.7}$	82	19	0	0	—
PARA-adeno 1	$10^{2.8}$	$10^{6.8}$	81	40	14	35	22–29
Adenovirus 2	—	$10^{6.0}$	140	35	0	0	—
PARA-adeno 2	$10^{4.0}$–$10^{5.6}$	$10^{6.5}$–$10^{7.6}$	340	128	24	19	12–34
Adenovirus 5	—	$10^{5.9}$	107	40	0	0	—
PARA-adeno 5	$10^{5.3}$	$10^{6.0}$	110	51	3	6	8–10
Adenovirus 6	—	$10^{5.8}$	84	45	0	0	—
PARA-adeno 6	$10^{2.4}$	$10^{5.8}$	81	44	14	32	13–31

* Obtained by plaque assay in green monkey kidney cells coinfected with a helper adenovirus.

‡ Obtained by plaque assay in human embryonic kidney cells.

§ Based on number weaned.

previously established that the tumors induced by PARA–adenovirus 2 (7, 25) and PARA–adenovirus 7 (1, 7) were virus-free. Extracts prepared from tumors induced by PARA–adenovirus types 1 and 6 failed to yield recognizable virus particles when examined in the electron microscope. A total of 14 (of 19 attempted) of these tumors were trypsinized and established in cell culture. Attempts to isolate either SV40 or adenoviruses from these cultures by a variety of techniques failed. These attempts included (*a*) disruption of the tumor cells followed by plating on GMK and HEK cells and, (*b*) mixing the tumor cells with GMK and HEK cells and growing them together.

Tumors were characterized histopathologically as previously described (10)

and analyzed for virus T antigens. The results with tumors induced by PARA-adenovirus types 2 and 7 have already been described (7, 10). As with tumors induced by PARA–adenovirus 2 and PARA–adenovirus 7, most (5 of 6) of the PARA–adenovirus 1 tumors examined were found to synthesize SV40 T antigen (Table III). Correspondingly, 10 of 13 of the hamster sera examined in this group contained antibody against SV40 T antigen. The number of tumors synthesizing detectable levels of adenovirus T antigen was lower (2 of 6 exam-

TABLE III

Immunofluorescence Analysis of PARA–Adenovirus 1 Tumor Cells and Hamster Sera

Type of tumor	No.	Cells			Serum		
		No. examined	SV40 T antigen	Adeno T antigen	No. examined	SV40 T antibody	Adeno T antibody
Adenovirus	5	1	1	1	5	3	2
SV40	3	2	2	1	3	3	3
Mixed	4	2	1	0	4	3	2
Atypical	1	1	1	0	1	1	0
Total.....	13	6	5	2	13	10	7

TABLE IV

Immunofluorescence Analysis of PARA–Adenovirus 6 Tumor Cells and Hamster Sera

Type of tumor	No.	Cells			Serum		
		No. examined	SV40 T antigen	Adeno T antigen	No. examined	SV40 T antibody	Adeno T antibody
Adenovirus	5	3	2	0	5	3	2
SV40	0	—	—	—	—	—	—
Mixed	5	5	2	0	5	4	5
Atypical	1	0	—	—	1	0	0
Total.....	11	8	4	0	11	7	7

ined) and 7 of 13 sera analyzed contained antibody to adenovirus T antigens. The ratio of adeno-like tumors to those exhibiting the morphology of SV40 tumors or those containing elements of both types is also similar to those previously described with PARA–adenovirus 2 and PARA–adenovirus 7 tumors (7, 10).

Tumors induced by PARA–adenovirus 6 were also analyzed (Table IV). Surprisingly, none of the tumors in this series exhibited the fibroblastic SV40-like morphology. Five of the tumors were epithelioid-like adenovirus tumors and five contained both elements. Only 4 of 8 tumors contained detectable

levels of SV40 T antigen with 7 of 11 sera containing the corresponding antibody. Seven sera also contained antibody against adenovirus T antigen, but none of the tumors examined had detectable quantities of the corresponding T antigen. Again, this is similar to previous results which have revealed that, in the adenovirus system, detection of the T antibody is more sensitive than detection of T antigen.

None of the cells examined contained SV40 V or adenovirus V antigen. None

TABLE V

Effect of Virus Immunization on Production of Tumors in Weanling Hamsters by SV40-Transformed Cells

Immunizing virus	Plaque-forming units per immunization*		No. animals with tumors/ No. animals challenged	No. of cells in one TPD_{50}‖
	PARA‡	Adenovirus§		
None	—	—	31/32	<100
SV 40	—	$10^{5.0}$	4/32	100,000
Adenovirus 1	—	$10^{6.4}$	22/32	500
Adenovirus 5	—	$10^{5.0}$	23/31	390
Adenovirus 6	—	$10^{4.2}$	29/32	<100
Adenovirus 7	—	$10^{5.0}$	27/32	100
Adenovirus 14	—	$10^{6.3}$	28/32	100
Adenovirus 21	—	$10^{4.2}$	23/32	390
PARA–adenovirus 1	$10^{3.3}$	$10^{6.4}$	18/31	1,500
PARA–adenovirus 5	$10^{4.3}$	$10^{5.0}$	4/32	100,000
PARA–adenovirus 6	$10^{2.2}$	$10^{4.2}$	11/32	15,000
PARA–adenovirus 7	$10^{4.2}$	$10^{5.0}$	8/28	25,000
PARA–adenovirus 14	$10^{4.2}$	$10^{6.3}$	8/32	31,000
PARA–adenovirus 21	$10^{4.1}$	$10^{4.2}$	0/32	>100,000

* Three inoculations given at weekly intervals.

‡ Obtained by plaque assay in green monkey kidney cells coinfected with a helper adenovirus.

§ Obtained by plaque assay in human embryonic kidney cells.

‖ Final readings taken 12 weeks after inoculation of the challenge cells.

of the sera from tumor-bearing hamsters contained antibodies to SV40 V antigen nor did these sera contain antibodies capable of neutralizing SV40 virus. However, a number of sera from animals bearing tumors induced by PARA–adenovirus types 1 or 6 contained neutralizing antibodies against the homologous, but not heterologous, adenovirus. Development of these antibodies was presumably stimulated by the original inoculum.

Effect of Immunization with PARA-Adenoviruses on Transplantability of Cells Transformed by SV40.—It had been previously shown that immunization

of weanling hamsters with PARA–adenovirus 7 (6) or with PARA–adenovirus 2 (26) confers protection to hamsters against challenge with cells transformed by SV40. It was postulated that failure to enhance oncogenic potential of the adenoviruses in the weakly oncogenic group by the addition of PARA (Table I) might be due to the inability of these viruses to enter the hamster cells efficiently to effect transformation. Such failure should also be reflected by decreased protection against the SV40-transformed cells after immunization of the hamsters with the same viruses.

To test this hypothesis, groups of weanling hamsters were inoculated with PARA-adenoviruses or with the parent PARA-free serotypes. The immunized animals were challenged 1 wk after the final (3rd) inoculation with 10-fold increments of H-50 cells. The immunizing dose and the number of cells per one TPD_{50} for each group at the termination of the experiment 12 wk post-challenge are shown in Table V.

The H-50 cells were highly oncogenic. Without prior immunization, tumors developed in 31 of 32 hamsters with less than 100 cells required for one TPD_{50}. Vaccination of hamsters with SV40 protected them against cells transformed by the homologous virus and 100,000 cells were therefore necessary for one TPD_{50}.

None of the parent PARA-free adenovirus serotypes tested (types 1, 5, 6, 7, 14, and 21) were capable of protecting hamsters against the H-50 cells. The number of cells required for one TPD_{50} ranged from less than 100 (for adenovirus 6) to 500 with adenovirus 1. However, animals inoculated with various PARA serotypes were protected against challenge with cells transformed by SV40. The most effective immunizing populations were PARA–adenovirus 5 and PARA–adenovirus 21; 100,000 cells or more were required for a TPD_{50} after inoculation with these populations. PARA–adenovirus types 6, 7, and 14 also protected the animals but to a lesser extent than SV40 or the other PARA populations tested. The results with PARA–adenovirus 7 were very similar to those previously reported from this laboratory (5) and the results for PARA–adenovirus types 5 and 21 resemble those previously reported with PARA–adenovirus 2 (26). PARA–adenovirus 1 was relatively ineffective in this series in protecting the animals against H-50 cells. The lower levels of protection obtained with PARA–adenovirus 1 and PARA–adenovirus 6 may reflect the somewhat lower titers of PARA being carried by those populations. It should be pointed out that the amount of adenovirus (as measured by ability to form plaques in HEK cells) was constant within various serotypes tested.

The time of tumor development in hamsters immunized with PARA-adenoviruses or the parent serotypes after challenge with H-50 cells is shown in Figs. 1 and 2. The TPD_{50} values were calculated at 2 wk intervals. The observed TPD_{50} remained constant (required more than 100,000 transformed cells) throughout the duration of the experiments for groups of hamsters vac-

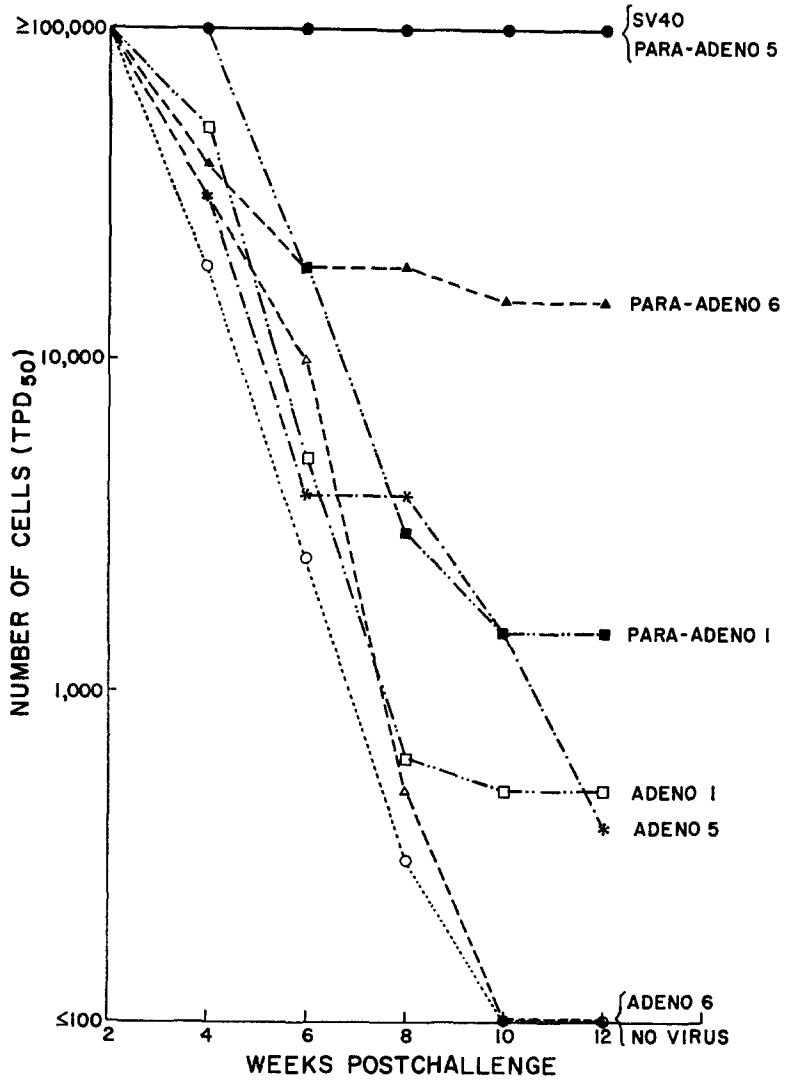

FIG. 1. Development of tumors (expressed as the number of cells required for one TPD_{50}) following challenge of hamsters with cells transformed by SV40 (H-50 cell line). Hamsters were previously immunized with previously nononcogenic adenovirus serotypes (1, 5, or 6) or with transcapsidant populations of these serotypes carrying a defective SV40 genome (PARA). Controls include nonimmunized animals and animals inoculated with SV40 virus.

cinated with SV40, PARA–adenovirus 5 (Fig. 1) and PARA–adenovirus 21 (Fig. 2). Protection afforded by PARA–adenovirus 6 (Fig. 1) and PARA–adenoviruses 7 and 14 (Fig. 2) dropped between the 4th and 6th wk and then remained fairly constant. When the parent adenovirus serotypes were used as

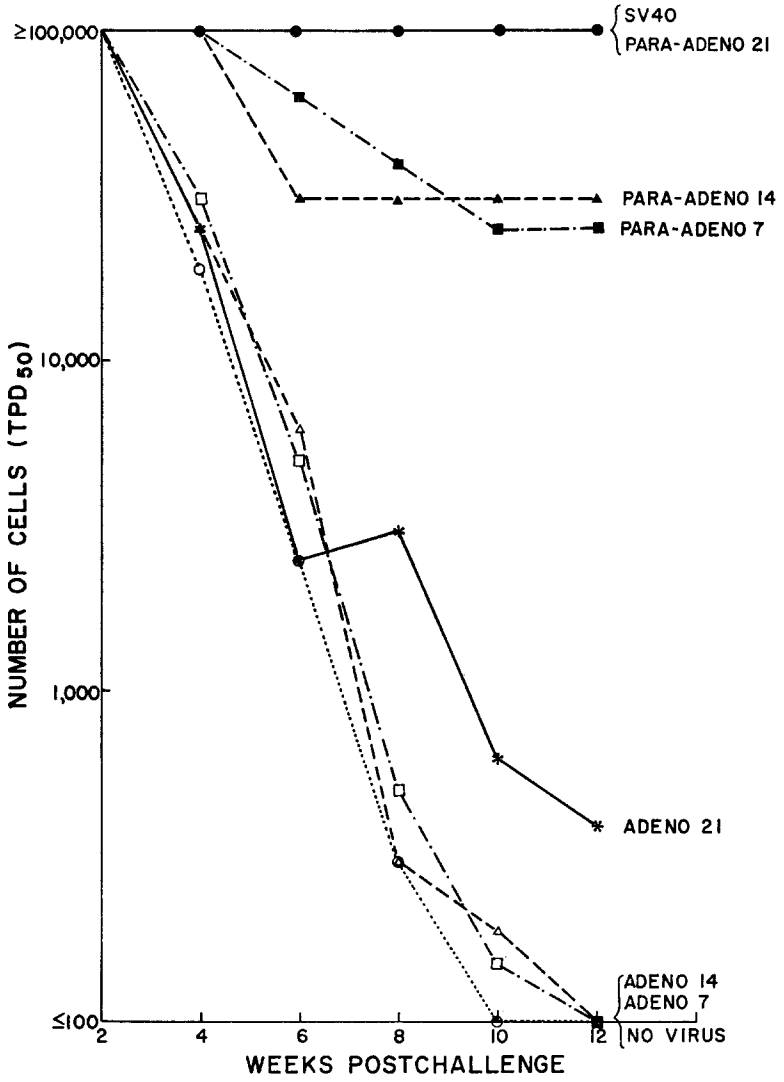

FIG. 2. Development of tumors (expressed as the number of cells required for one TPD_{50}) following challenge of hamsters with cells transformed by SV40 (H-50 cell line). Hamsters had been previously immunized with weakly oncogenic adenovirus serotypes (7, 14, or 21) or with transcapsidant populations of these serotypes carrying a defective SV40 genome (PARA). Curves for nonimmunized animals and for animals immunized with SV40 virus are redrawn from Fig. 1 for purposes of comparison.

immunizing agents, tumors continued to form so that the TPD_{50} values dropped sharply for the first 10 wk. Previous experience with the H-50 cells had revealed that new tumors do not develop after 12 wk following challenge of the animals with these cells. It is therefore apparent from these results that immunization with PARA-adenoviruses confers significant immunity to hamsters against challenge with H-50 cells, but that immunization with the PARA-free parent adenovirus serotypes does not; this immunity was obtained with adenoviruses carrying PARA that were oncogenic as well as with those that were not rendered oncogenic by the additon of the defective SV40 genome being carried by PARA.

DISCUSSION

The original demonstration by Huebner and his colleagues (1) that a human adenovirus type 7 was capable of inducing tumors in hamsters having the antigenic characteristics of SV40-induced tumors led to detailed analyses of the adenovirus type 7 population carrying the SV40 information. It was soon found that this population was morphologically and antigenically homogeneous (4, 5, 27, 28); that is, all of the nucleic acid of the virus population was encased in adenovirus capsids. However, the population did consist of two genotypes, one composed of the adenovirus type 7 DNA and the other including defective SV40 (8, 9, 15). It was also observed that the SV40 genome carried by the virus population contained the markers for the induction of SV40 T antigen (4, 5) as well as for SV40-specific transplantation antigens (6) and that acquisition of this defective information enabled human adenoviruses to replicate in simian cells (2, 3, 12, 15, 28). Transfer of the defective SV40 information from the parent adenovirus type 7 to heterologous adenovirus serotypes by a process termed transcapsidation (8, 9) resulted in the acquisition of oncogenic potential when the SV40 information was carried by adenovirus type 2 (7, 10, 11). It was therefore anticipated that transfer of this information to other adenovirus serotypes would result in a similar acquisition of oncogenic potential.

This study clearly reveals that such is not the case and that oncogenic potential is acquired only by certain adenoviruses carrying the defective SV40 genome in PARA. Since, as also revealed in this study, all PARA populations tested appear capable of inducing transplantation immunity to cells transformed by SV40, it would appear that failure to enter the hamster cell is not the reason for lack of oncogenic potential. It is, however, possible that the adenovirus-PARA populations can enter cells of the weanling hamster to transform them so that subsequent challenge of the immunologically competent animal with SV40 transformed cells is aborted. The same virus populations may, however, be unable to enter hamster embryonic cells to effect transformation of those cells. Preliminary studies carried out to determine the latter have revealed that most adenovirus populations adsorb and penetrate very

poorly into hamster embryonic cells, but no clear-cut difference has been observed between those populations that are oncogenic and those that are not oncogenic following the acquisition of PARA.

Freeman et al. (29) have recently reported that adenovirus type 2 can transform primary rat embryo cells. Based on this observation and on serologic cross-reactions between various virus-induced tumor antigens, Huebner (personal communication) has suggested that adenovirus types 1, 2, 5, and 6 represent a subgroup of adenoviruses. This subgroup has been characterized earlier by its hemagglutination pattern (22), by possession of DNA of high GC content (24), and by hybridization results between viral DNA and tumor mRNA (30). The acquisition by these same serotypes of oncogenic potential when carrying PARA supports their being grouped together. Other adenoviruses will have to be tested to determine the limits of this grouping; such tests of oncogenic potential after the transfer of PARA to other adenovirus serotypes are presently under way in our laboratory. However, the fact that the acquisition of PARA by some nononcogenic adenoviruses renders them oncogenic, while other weakly oncogenic adenoviruses belonging to the subgroup with DNA of intermediate GC content are not rendered more oncogenic, is of fundamental importance, and the systems described in this study will require additional detailed investigation.

SUMMARY

The acquisition of the defective SV40 genome by a variety of human adenovirus serotypes by the process of transcapsidation has resulted in the addition of oncogenic potential for newborn hamsters to the previously nononcogenic adenovirus types 1, 2, 5, and 6. These serotypes have previously been grouped together by the high GC content of their DNA.

Transcapsidation of the SV40 genome to weakly oncogenic adenovirus types 3, 14, 16, and 21 has failed to increase their oncogenic potential although the parent adenovirus type 7 carrying PARA is highly oncogenic. These serotypes belong to the group possessing a DNA of intermediate GC content.

All the PARA-adenovirus populations, even those that were nononcogenic, were able to induce SV40 transplantation immunity and therefore carry the SV40 transplantation marker as well as the marker for synthesis of SV40 tumor or T antigen.

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