Variation in Response of Syrian Hamster Lung Cells to Complete or Defective SV40 (PARA)[1] (35130)

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It was shown by the use of the immunofluorescence technique in 1964 that cells transformed by SV40 contained a new intranuclear antigen designated the tumor (T) antigen (1, 2). Later reports showed that newborn Syrian hamster lung cells appeared to transform following exposure to SV40 but did not develop detectable amounts of T antigen (3, 4).

A defective SV40 genome was subsequently demonstrated to be associated with a simian cell-adapted strain of adenovirus type 7 (5–7). The defective SV40 genome encased in an adenovirus capsid was named "PARA" (8) and was shown to carry the information for the synthesis of SV40 T antigen following either productive infection or transformation of cells (5–7, 9).

It was of interest to determine whether Syrian hamster lung cells responded to exposure to PARA the same as they did to SV40, particularly with respect to the synthesis of virus-specific antigens. This report shows that, in contrast to cultures exposed to parental SV40, the lung cells transformed by several different variants of PARA do contain detectable levels of SV40 T antigen.

Materials and Methods. Cells. Primary cultures of lung cells were prepared from a weanling male of the LSH inbred strain of Syrian hamsters obtained from Lakeview Hamster Colony, Newfield, New Jersey. The lungs were removed aseptically, minced, and trypsinized at 37° with a 0.25% (w/v) solution of trypsin.

Uninoculated and SV40-inoculated cultures were grown in Eagle's basal medium supplemented with 10% fetal bovine serum, 100 units/ml of penicillin, 100 μg/ml of streptomycin, 50 units/ml each of Mycostatin and Fungizone, and 0.075% sodium bicarbonate. Cells inoculated with PARA-adenovirus hybrid populations were grown in low calcium Eagle's minimal essential medium (10) supplemented as above. The low calcium medium was subsequently found not to be a requirement for the growth of the PARA-transformed cells.

Viruses. The Baylor reference strain of SV40 was used (11). It had been passed seven times in primary green monkey kidney (GM K) cells, plaque-purified in CV-1 cells, and then passed eight times in CV-1 cells. It had a titer of 1×10^7 plaque-forming units (PFU)/ml.

The L.L. strain of adenovirus 7, described elsewhere (7), was plaque-purified twice in GMK cells and clonal lines of virus were derived (12). The virus clones used in this study had been passed three times in GMK cells and were designated PARA (10nT)-adenovirus 7, PARA (39nT)-adenovirus 7, PARA (73nT)-adenovirus 7, PARA (1cT)-adenovirus 7 and PARA (2cT)-adenovirus 7. The titers of PARA in these stocks ranged from $0.5–5.0 \times 10^5$ PFU/ml. PARA (1cT)-adenovirus 7 and PARA (2cT)-adenovirus 7 are unique in that they induce the synthesis of SV40 T antigen in the cytoplasm of cells (12, 13). PARA-adenovirus 16 was prepared by transcapsidation in a previous study (14), had undergone three passages in GMK cells, and had a PARA titer of 6×10^4 PFU/ml.

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TABLE I. Derivation of Cell Lines from Syrian Hamster Lung Cells Transformed *in Vitro* by SV40 and PARA-adenoviruses.[a]

Cell line	Transforming virus	MOI SV40 or PARA (PFU/cell)	Days pi		Morphology
			First passage	Second passage	
SHL-S	SV40	26	5	12	Fibroblastic
SHL-7 (10)	PARA (10nT)-Ad7	3.0	13	34	Fibroblastic
SHL-7 (39)	PARA (39nT)-Ad7	6.3	13	35	Fibroblastic
SHL-7 (73)	PARA (73nT)-Ad7	1.3	12	28	Fibroblastic
SHL-7 (1cT)	PARA (1cT)-Ad7	9.0	24	42	Fibroblastic
SHL-7 (2cT)	PARA (2cT)-Ad7	1.5	12	47	Fibroblastic
SHL-16	PARA-Ad16	0.06	5	14	Fibroblastic

[a] MOI = multiplicity of infection; pi = postinoculation.

Immunofluorescence techniques. The procedure used for the detection of SV40 and adenovirus T antigens has been described in detail (12). SV40 surface (S) antigen was detected on viable cells by the method of Tevethia *et al.* (15).

Transformation experiments. Two approaches were used to infect the cells in the transformation experiments. Method 1: Following the method of Duff and Rapp (16), secondary cultures of hamster lung cells were trypsinized and suspended in Eagle's medium, mixed with an equal volume of virus suspension, and shaken for 3 hr at 37°. At that time 0.4-ml aliquots of the suspension were distributed into 60-mm plastic petri dishes and 5 ml of complete growth medium were added to each dish. The cultures were incubated at 37° in an atmosphere of 5% CO_2 with fluid changes every 2–3 days. Method 2: Nonconfluent monolayers of fourth passage cultures of lung cells in 8-oz bottles were exposed to 1 ml of virus suspension. After a 3-hr adsorption period at 37° with intermittent shaking, 25 ml of growth medium were added/bottle.

Results. Growth characteristics of control and inoculated Syrian hamster lung cells. Control uninoculated lung cells (SHL) showed active proliferation for a period of approximately 2 months after explantation and could be passed weekly. After 2 months, the cells reached a stationary phase, could not be subcultured, and gradually died out.

No apparent cytopathic effects (CPE) were observed when the SHL cells were exposed to SV40 at a multiplicity of infection (MOI) of 26 PFU/cell (Table I). The exposed cells grew quickly and the culture medium rapidly became intensely acidified. At passage 16, the cell line (SHL-S) went into crisis, but recovered satisfactorily. The cells retained a fibroblastic morphology (Table I) and even at 7 months postinoculation (pi) did not exhibit any tendency to pile up.

All the cultures of SHL cells inoculated with different clonal lines of PARA-adenovirus hybrid populations at the MOI shown in Table I exhibited transitory adenovirus CPE. However, all the cultures recovered and were successfully subcultured at the intervals pi indicated in Table I. All the cells lines which were established from the PARA-infected cultures displayed a similar pattern of rapid and disorganized growth, intense acidification of the culture medium, and retention of a fibroblast-like morphology.

Presence of SV40 virus-specific antigens in transformed Syrian hamster lung cells. The PARA- and SV40-transformed hamster lung cell lines were tested by immunofluorescence for the presence of SV40 T and S antigens as well as adenovirus T antigen (Table II). The six cell lines derived from cultures transformed by different clonal lines of PARA all contained SV40 T antigen. As shown in Fig. 1, the intranuclear T antigen reaction observed with the PARA-transformed lung cells is typical of that seen in the majority of SV40-transformed cells. The fibroblastic morphology of the transformed lung cells is also apparent in Fig. 1.

TABLE II. Antigenic Analysis of Syrian Hamster Lung Cells Transformed *in Vitro* by PARA-adenoviruses and SV40.[a]

Cell line	SV40 T antigen		SV40 S antigen		Adeno T antigen	
	Passage	Result	Passage	Result	Passage	Result
SHL-7 (10)	4	+	7	+	11	0
SHL-7 (39)	3	+	7	+	8	+
SHL-7 (73)	4	+	12, 15	+	23	0
SHL-7 (1cT)	5	+ (cyto)	11	+	10	0
SHL-7 (2cT)	4	+	10, 12	+	14	0
SHL-16	10	+	11	+	16	+
SHL-S	5, 9, 13	0	9	0	Not done	

[a] + = presence of antigen, 0 = absence of antigen, cyto = cytoplasm.

In one cell line, SHL-7(1cT), the SV40 T antigen was spread diffusely throughout the cytoplasm with no reaction detected in the nucleus.

Repeated testings of the SV40-transformed cell line, SHL-S, with the same immunofluorescence reagents failed to reveal any SV40 T antigen positive cells (Table II). This observation is in agreement with that of previous reports (3, 4, 17).

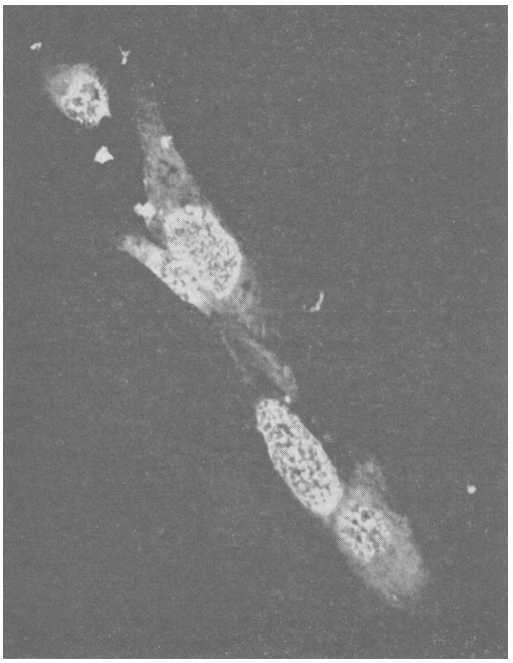

FIG. 1. Immunofluorescence detection of SV40 T antigen in Syrian hamster lung cells transformed by PARA (39nT)-adenovirus 7. Note fibroblastic morphology of the cells; ×400.

The transformed cell lines were first tested for the presence of SV40 S antigen between passages 7 and 12 (Table II). All the PARA-transformed lines contained this antigen at the cell membrane. However, S antigen was not detected in the lung cells exposed to SV40. This absence of S antigen correlates well with results obtained previously (18) with 3 of 6 SV40-transformed hamster lung cell lines.

Since adenovirions were present in all the stocks of PARA, the transformed cell lines were also assayed for the presence of adenovirus T antigen (Table II). Only the SHL-7(39) and SHL-16 cell lines contained detectable levels of adenovirus T antigen. However, animals bearing tumors induced by the SHL-7(73) and SHL-7(2cT) cells have yielded adenovirus T antibody, indicating that at least these two cell lines also contain adenovirus information.

Discussion. This paper demonstrates that PARA (defective SV40) can successfully transform Syrian hamster lung cells *in vitro*. Of particular interest is the observation that the lung cells transformed by PARA all contained detectable levels of SV40 T antigen. This is in contrast to cultures of lung cells exposed to parental SV40 which transformed but failed to synthesize SV40 T antigen. The response of SHL cells to complete and defective SV40 are compared in Table III. Four separate reports, originating from 3 different laboratories, have established that SHL cells consistently acquire an infinite life *in vitro* following exposure to SV40 but do not synthesize detectable amounts of T antigen.

TABLE III. Comparison of Response of Syrian Hamster Lung Cells to SV40 or PARA.

Virus	No. of lines		No. of lines with SV40 antigens		Ref.
	Exposed	Transformed	T	S	
SV40	6	6	0	3	(3, 18)
SV40	1	1	0	ND[a]	(4)
SV40	1	1	0	ND	(17)
SV40	1	1	0	0	This report
PARA	6	6	6	6	This report

[a] ND = not done.

Some lines contain SV40 S antigen and others do not. In contrast, all 6 cultures exposed to variants of PARA in this study transformed and synthesized detectable levels of T and S antigens.

Although definitive evidence that SV40 is actually responsible for the observed transformation of the SHL-S cells is still lacking, the cells did undergo transformation following virus exposure, whereas companion control cultures did not. An increased growth potential was acquired by BHK21 cells after exposure to SV40 DNA in the absence of synthesis of T antigen (19), strengthening the possibility that a virus–cell relationship can occur which does not result in the synthesis of T antigen.

Several possible explanations are prompted by the difference observed in the response of lung cells to SV40 and PARA: (i) It has been shown that T antigen synthesis induced by PARA has a resistance to interferon more characteristic of adenoviruses than the comparative interferon sensitivity of T antigen induced by SV40 (20). This is probably due to the fact that a segment of adenovirus 7 DNA is covalently linked to the defective SV40 DNA in PARA (21, 22). Therefore, a superior capacity for interferon production could explain the variable response of Syrian hamster lung cells to T antigen synthesis induced by SV40 or PARA. In fact, organs containing phagocytic cells form interferon more rapidly than other tissues (23) and lung tissue contains a differentiated macrophagic type cell (24); (ii) we have shown by the use of lung cells from hybrid animals that a haploid chromosome complement from the Romanian hamster species alters the response of the haploid Syrian hamster genome to SV40 such that T antigen is synthesized following transformation (25). Accordingly, the observed results might be a consequence of some contribution the cellular genome makes in determining the type of transformation, possibly at the level of regulation of transcription or translation of a persisting viral genome; (iii) the encasement of the defective SV40 genome in an adenocapsid and the presence of complete adenovirions in the PARA inocula may have, in some way, altered adsorption, penetration, uncoating, or subsequent events following virus inoculation; (iv) while the PARA genome probably does persist in the transformed cultures, it is possible that, in contrast, SV40 actually mediated the transformation observed in the SHL-S cells, but no longer persists. Evidence currently available does not allow us to distinguish between the possible explanations outlined above.

This study confirms previous observations [(26), Richardson and Butel, in preparation] that SV40 T antigen can be localized in the cytoplasm of transformed cells. It also demonstrates the variable response that can be observed with differentiated host cells with respect to the expression of virus-specific antigens after exposure to viruses containing similar genetic information. This observation is pertinent in view of attempts to detect virus-specific antigens as an approach to demonstrating a viral etiology of human tumors.

Summary. Adult Syrian hamster lung cells were exposed to six clonal lines of PARA (defective SV40) as well as to parental SV40. All the exposed cultures transformed. SV40

T and S antigens were synthesized in all the cells transformed by PARA, but could not be detected in the culture which transformed following exposure to SV40.

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