Activation of Type C RNA Tumor Virus Expressions in Tumors Induced by Cell Cultures Transformed by Polyoma Virus[1] (37558)

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We reported accelerated transformation by DNA viruses (SV40 and polyoma) in rat cells chronically infected with a type C RNA virus (1, 2) compared to uninfected rat cells. A possible explanation for the observed enhancement was based on the oncogene hypothesis of Huebner and associates (3–5) which postulates that the transforming oncogene known to be present in the type C RNA tumor viruses and normal mouse, hamster and chicken cells is also present in rat cells as an endogenous type-C RNA virus genome, and that the transformation induced by polyoma virus in normal cells and the accelerated transformation in the type C RNA virus infected cells determined by endogenous and injected type C RNA oncogenes respectively.

Recently we found the group-specific (gs) antigen expressions of type C RNA tumor viruses in tumors induced by mouse and hamster embryo cultures transformed by polyoma virus. Since all cells of mice, hamster, rats, and chickens have now been shown to contain the complete genome of the RNA tumor viruses, we suggest that polyoma virus "switches on" expressions of the type-C RNA tumor viruses in mice and hamster and thus the DNA viruses operate (only in heterologous species) to activate the oncogenes of the RNA tumor viruses.

Materials and Methods. Virus. Polyoma virus as obtained from Dr. K. K. Takemoto, National Institute of Allergy and Infectious Diseases, NIH (originally from Dr. B. E. Eddy, NIH) and was grown in suckling mouse kidney cultures by the method of Winocour (6). Its infectivity titer was 5×10^8 PFU/ml in mouse embryo cells. The virus preparation was treated with ether (20%) before being used in this experiment and did not contain detectable gs complement-fixing (CF) antigens of the murine leukemia–sarcoma virus complex (7, 8).

Cell culture and media. Eagle's minimal essential medium supplemented with 10% fetal bovine serum, 2 m*M* glutamine, 100 units/ml of penicillin and 100 μg/ml of streptomycin was used for growing and maintaining cells. Normal mouse embryo (ME) cell lines used in this study were established from primary NIH Swiss ME cells (NIH-ME) (9). Primary cultures of NIH-ME cells were obtained from Microbiological Associates, Inc., Bethesda, Maryland. Normal hamster embryo (HE) cell lines (HE ER-9) were established from primary NIH golden HE cells, and the 9th subculture level was used. All cell lines were cultivated in a 5% CO_2 atmosphere at 37°.

Complement fixation. Complement-fixation tests were carried out by the microtiter technique described for tumor "T" and viral antigen studies (10). Titers were recorded as reciprocals of the highest dilution giving 3+ to 4+ fixation of 1.8 units of complement. Cell pack preparations for CF testing were made as previously described (7). Broadly reactive rat antisera used for detecting gs antigens (both gs-1 and gs-3) in the CF test and complement fixation tests for murine leukemia virus (COMUL test) (7, 8) were obtained from Fischer rats carrying transplanted sarcomas induced by the Moloney sarcoma virus (M-MSV). An antiserum prepared in guinea pigs with purified gs anti-

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gen of the viruses of murine leukemia sarcoma group (11, 12) was also used. Polyoma "T" antisera was obtained from hamsters bearing polyoma tumors.

Results and Discussion. The first experiment was done with NIH-ME (R-616U) cultures at the 17th subculture. Three weeks after infection the cells were further subcultured and cell lines were established. Cell passages were thereafter generally made once a week when cultures were fully confluent. Patches of small dark cells appeared on sheets of normal cells approximately 5–7 days after infection. The number of such cells progressively increased until most of the cells were involved, and many then sloughed off from the plate. Small, densely opaque foci were first observed 14–17 days after infection. The number of altered cells increased and weekly transfer of the cells became possible. The transformed cells consisted of many layers of cells having stellate or triangular shape and they grew at random, criss-crossing one another and mitotic figures were abundant. The appearance of giant cells was also characteristic of these cells (Fig. 1A). Cell pack preparations of polyoma virus transformed cells did react in CF test with polyoma tumor ("T") and but did not react with viral antibody. The transformed cells did not react with MSV rat antiserum. Tumors were readily produced in newborn NIH Swiss mice by inoculation of the transformed cells (Table I). When 10% tumor extracts were tested in CF test for gs antigen, all the tumors contained antigens reactive with both MSV rat and guinea pig antiserum. The guinea pig antiserum is reactive specifically with the gs-1 component of the type C RNA viruses. Polyoma virus "T" antigen was demonstrated in CF test; but polyoma viral antigens were not detected (Table I). The cultured tumor cells contained both gs antigen and polyoma "T" antigen.

In a second experiment, another Swiss NIH-ME line (R616 R) at the 20th subculture was used. Two weeks after infection, the cells were subcultured and cell lines were established. The results were similar to those presented above (Table I). Our attempts to isolate infectious virus (polyoma or type C virus) from the CF positive tumor preparations in *in vitro* COMUL test have been negative.

The third experiment was done with an early passage of HE cells. Two weeks after infection, the cells were passaged and cell lines were established. By 3 wk the number of infected cells increased about threefold as compared to normal cells, and they grew as piled up whorls. The transformed cells did not react with MSV rat and polyoma viral antiserum but did react with polyoma "T" antiserum. Tumors were readily produced when the transformed cells were transplanted into newborn NIH hamsters (Table II). The tumors were progressive and sectioned and diagnosed histologically as sarcomas by Dr. L. Rabstein. Both the cultured tumor cells and hamster tumors contained antigens reactive with hamster leukemia virus-specific guinea pig antiserum. Polyoma "T" antigens were also demonstrated in CF test: but polyoma viral antigens were not detected (Table II).

Of special interest in the present study was activation of type C RNA tumor virus expressions in tumors induced by mouse and hamster cell cultures transformed by polyoma virus. When polyoma virus transformed NIH Swiss mouse cells were transplanted into newborn Swiss mice the tumors revealed large amounts of species-specific murine leukemia virus gs antigen but were negative for infectious virus. Since the normal NIH Swiss mouse is negative for infectious viruses, and apparently cannot under any conditions made infectious virus (13), the evidence favors amplification in the tumor exclusive of RNA tumor virus expressions induced by the polyoma virus. We have often observed the type C RNA virus expressions in tumors induced by spontaneously transformed NIH Swiss mouse embryo cells after long-term *in vitro* cultivation (Rhim *et al.*, unpublished data.) Similar findings were observed in polyoma virus hamster cells system as reported herein. Polyoma virus transformed hamster embryo cells regularly led to hamster leukemia virus gs antigen after transplantation into newborn virus-free hamsters. Perhaps, this is also true in rat cell system since polyoma virus trans-

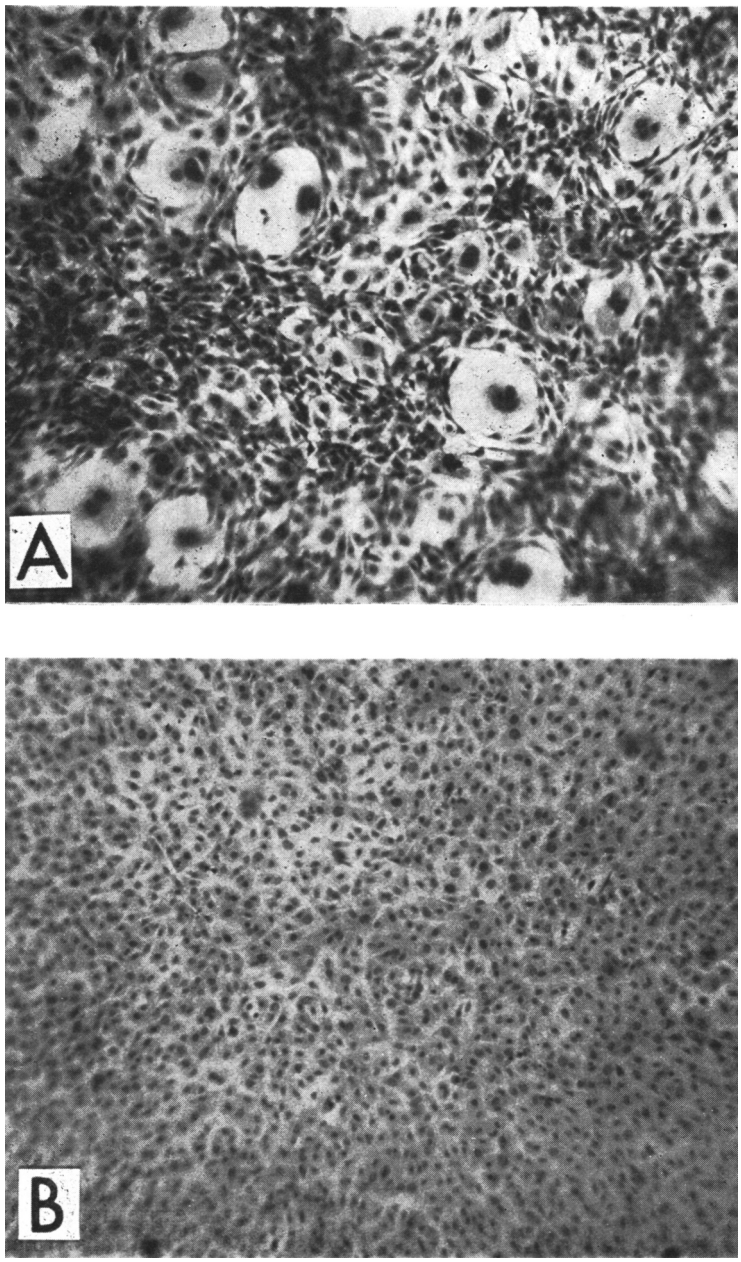

FIG. 1A. Culture of NIH Swiss mouse embryo (NIH-ME) cells. (Giemsa Stain) (× 40) passage 9. Polyoma virus transformed NIH-ME cells, spindle-shaped, giant cells, loss of cell orientation, piling up of cultures.

FIG. 1B. Culture of NIH Swiss mouse embryo (NIH-ME) cells. (Giemsa Stain) (× 40) passage 9. Normal, uninfected NIH-ME cells.

formed rat cells and tumors induced by the transformed cells expressed CF antigen reactive with MSV rat antiserum (14). In addition, we recently observed the expressions of rat-specific type C virus antigens in rat cells transformed *in vitro* spontaneously or by polyoma virus (Rhim *et al.*, unpublished data).

TABLE I. Presence of Complement-Fixing (CF) Antigen in Tumors Inoculated Subcutaneously with NIH Swiss Mouse Embryo Cells Transformed by Polyoma Virus (1×10^6 Cells Inoculated/Animal).

Expt no.	Donor (passage)	No. with tumor/ no. inoculated	Tumor	Subculture no. when CF tested	CF titers[a] against MSV Rat	MSV Guinea pig	Polyoma "T"	Polyoma "V"
1.	R-616u (p-9)	3/4 (17)[b]	Tumor #1	Primary	1:4	1:2	1:4	0
			Tumor #2	Primary	1:4	1:2	1:4	0
			Tumor #3	Primary	1:4	1:2	1:4	0
			Line 1:	2	0	ND	0	0
				3	1:2	ND	1:4	0
				5	1:2	ND	1:4	0
			Line 2:	Primary	1:2	ND	1:2	0
2.	R-616u (p-13)	1/3 (21)	Tumor	Primary	1:4	ND	1:4	0
3.	R-616R (p-15)	5/5 (6)	Tumor #1	Primary	1:2	ND	0	0
			Tumor #2	Primary	1:2	ND	1:2	0
			Line 1:	3	3	ND	1:2	0

[a] CF titers = reciprocal of dilution giving 3^+ to 4^+ fixation of 1.8 units of complement. $0 = < 2$. ND = not done; polyoma "T" = polyoma hamster tumor serum; polyoma "V" = polyoma mouse tumor serum.

[b] Day tumor first seen.

Activation of type C RNA viruses from tumors induced by cell cultures transformed by chemical carcinogens has recently been reported (15, 16). Since polyoma virus like SV40 and adenoviruses have not been shown to cause tumors in their natural hosts we speculated that these findings favored the hypothesis that neoplastic changes produced in heterologous cells and animals by these viruses are due to derepression or activation of the RNA virus genome. Thus, DNA viruses (polyoma and SV40 viruses) do interact with RNA tumor virus genomes like chemical carcinogens (15, 16) and the endogenous RNA tumor virus genome are responsible for cell transformation by "oncogenic" DNA tumor viruses (polyoma, SV40, adenoviruses, and herpes type viruses) as part of the viral DNA or the cell DNA and do participate in the transformation process of DNA tumor viruses as suggested previously (1, 2).

The data obtained indicate that polyoma virus can induce transformation in established mouse and hamster embryo cell lines. Transformation by polyoma virus was evidenced by: (a) morphological alteration with

TABLE II. Presence of CF Antigen in Tumors and Tumor Cell Lines Induced with Hamster Embryo Cells Transformed by Polyoma Virus: (1×10^6 Cells Inoculated/Animal).

Expt no.	Donor (passage)	No. with tumor/ no. inoculated	Specimens	CF titers[a] against Hamster leukemia virus guinea pig antiserum	Polyoma "T"	Polyoma "V"
1.	R-757 (p-6)	[b]6/6 (14)[c]	10% tumor extract	1:4	1:2	0
			Tumor cell line	>4	0	0
2.	R-757 (p-8)	4/4 (11)	10% tumor extract	1:4	1:2	0
			Tumor cell line	1:4	1:2	0

[a] CF titers: $0 = 2$.

[b] Tumors were diagnosed as sarcomas by Dr. L. Rabstein.

[c] Day tumor first seen.

foci formation; (b) presence of polyoma "T" antigen by a CF test. Transformation of mouse and hamster cells by polyoma virus has previously been reported (17–20). Our data indicate also that transformation by polyoma virus was facilitated by the use of established embryo cell lines instead of primary or secondary cultures, a finding previously reported in other systems (18, 19). We failed to obtain the polyoma virus transformation in mouse cells using primary or secondary cultures in several attempts.

Summary. NIH Swiss mouse embryo cells transformed by polyoma virus were negative for virus and group-specific (gs) antigens of type C RNA tumor virus. When the transformed cells were transplanted into subcutaneous tissues of newborn NIH Swiss mice the tumors were positive for gs antigens but negative for infectious virus. Similarly, polyoma virus transformed hamster embryo cells led to hamster leukemia virus gs antigen after transplantation into newborn virus-free hamsters. Since mouse and hamster cells have been shown to contain the complete genome of the RNA tumor viruses, we suggest that a DNA tumor (polyoma) virus transformation in cell cultures and expressed (or activated) gs antigen of type C RNA tumor virus in tumors may be related events.

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