Accelerated Transformation by Polyoma Virus in Rat Embryo Cells Infected with Rauscher Leukemia Virus[1] (35885)

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We recently reported accelerated transformation by simian virus 40 (SV_{40}) in rat embryo (RE) cells chronically infected with Rauscher leukemia virus (RLV) (1). In this communication we report similar effects of polyoma virus on RLV-infected RE cells. As with SV_{40}, infection with RLV led to accelerated transformation by polyoma virus compared to uninfected RE cells.

Materials and Methods. Virus. Polyoma virus was originally obtained from Dr. B. E. Eddy, Division of Biological Standards, National Institutes of Health and was grown in suckling mouse kidney cultures by the method of Winocour (2). 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 group specific (gs) complement-fixing (CF) antigens of the murine leukemia–sarcoma virus complex (3, 4).

Cell culture and media. Rat embryo lines. Rauscher leukemia virus-infected RE (F-119) and uninfected RE (F-111) cell lines used in this study were those established by Freeman *et al.* (5); the 39th and 40th subcultures were used. These normal appearing RE cultures, infected with RLV, have been shown to continuously replicate the virus and produce gs CF antigen. Production of complete virus was indicated by the presence of numerous budding C-type particles in electron micrographs and by the presence of infectious virus (3.5–4.0 logs of virus/ml) in the *in vitro* COMUL test (3, 4). However, neither C-type RNA virus nor gs antigens were detected in uninfected RE (F-111) cell line up to the 65th subcultures. Growth and maintenance medium consisted of 10% fetal bovine serum in Eagle's minimum essential medium with 2 m*M* glutamine and antibiotics.

Complement fixation. Complement-fixation tests were carried out by the microtiter technique described for tumor and viral antigen studies (6). Titers were recorded as reciprocals of the highest dilution giving 3+ to 4+ fixation of 1.8 units of complement. Cell pack preparations for gs antigen test were made from infected and uninfected RE cells as previously described (7).

Antisera. Rat antisera used in COMUL tests were obtained from Fisher rats carrying transplanted sarcomas induced by the Moloncy sarcoma virus (M-MSV). Polyoma "T" antisera was obtained from hamsters bearing polyoma tumors.

Transformation studies. Sixteen-ounce bottle cultures of RLV infected and uninfected RE cells were infected with 5×10^8 PFU of polyoma virus. The infected cultures were incubated for 3 hr at 37°, then washed three times with medium. Cells were then trypsinized and plated into petri dishes at different concentrations. The cultures were incubated at 37° under 5% CO_2 atmosphere with medium changes twice a week. Replicate cultures were stained with Giemsa and foci were counted. Transformation rates were then determined.

Results. The first series of experiments were done with RLV-infected RE (F-119) and uninfected RE (F-111) cultures both at 39th subculture. Eight to 10 days after infec-

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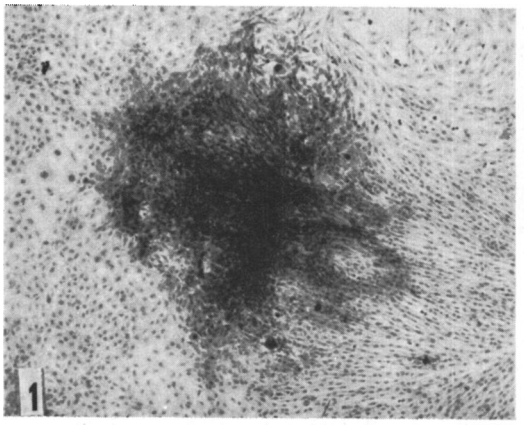

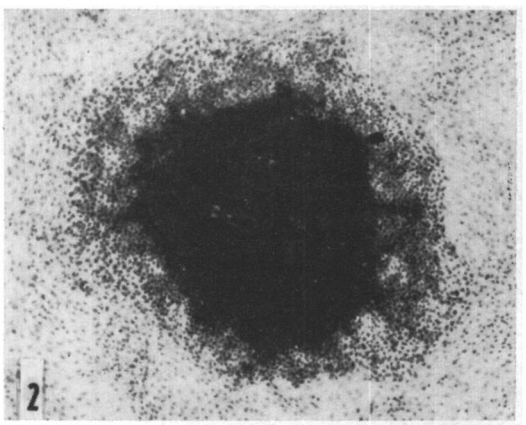

FIG. 1. A transformed focus in a culture seeded 14 days previously with polyoma-infected RLV-RE cells. Giemsa stain.

FIG. 2. A transformed focus in a culture seeded 14 days previously with polyoma-infected RE cells. Giemsa stain. The transformed cells form a dense defined colony in which the cells pile up on each other at random.

tion small, densely opaque foci were seen in the RLV-infected RE (F-119) cultures. Microscopic examination of these foci showed that they consisted of cells piled up in a multilayered, irregular array. The stained transformed foci could be readily distinguished from normal, nontransformed cells (Fig. 1). The transformed foci, however, did not all have the same gross morphological appearance.

In the RLV uninfected RE (F-111) cells, foci were first observed 11 to 13 days after infection. The foci consisted of well-defined aggregates of piled-up ovoid cells (Fig. 2) and they were larger and more sharply delineated from the surrounding fibroblast-like cells than those observed in the RLV-infected RE (F-119) cells. By the end of the third week after infection, numerous foci were also present in the RLV-infected RE cells; however, less foci were observed in the uninfected RE cells (Fig. 3). Transformation efficiency of polyoma virus in RLV-infected RE cells determined after 21 days of infection was 20 to 30 times greater than that obtained in RLV uninfected RE cells (Table I). Thus, increased transformation efficiency of polyoma virus in RLV-infected RE cells was evident.

During the second week after polyoma virus infection, cell pack preparations of both transformed RE cell lines reacted specifically in CF test with polyoma virus T specific antisera but did not react with polyoma viral antisera. Polyoma virus T antigen was also demonstrated in the transformed cells by fluorescent antibody (FA) test. In the CF test, RLV-infected RE cells transformed by polyoma virus reacted with MSV rat antiserum.

In a second experiment, RLV-infected RE (F-119) and uninfected RE (F-111) cells at the 40th passage were used. The results were similar: (i) Two types of foci were noted; and (ii) more foci were observed in the RLV-infected RE cells. (iii) Both RE cells transformed by polyoma virus reacted with polyoma T antisera but did not react with polyoma viral antisera. (iv) The RLV-infected RE cells transformed by polyoma virus reacted also with MSV rat antisera.

Discussion. Our studies indicate that polyoma virus can induce transformation in established cell lines of RLV-infected and uninfected RE cells, and that transformation rate was significantly increased in the RLV-infected RE cells; these findings were similar to those previously reported using SV_{40} virus (1). Transformation by polyoma virus was evidenced by: (i) morphological alteration with foci formation; (ii) presence of polyoma tumor antigen detected by CF and

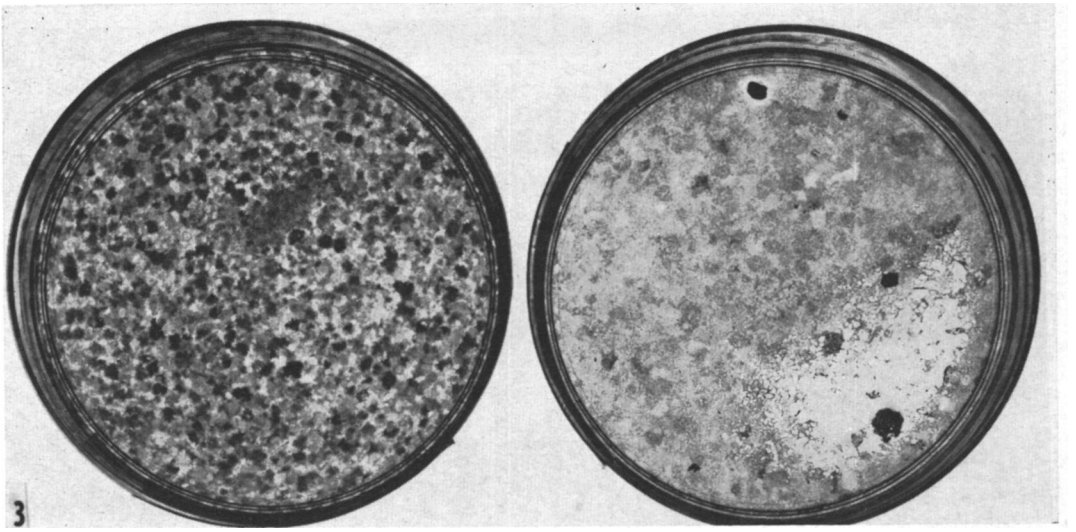

FIG. 3. Cultures of RLV-infected and uninfected rat embryo (RE) cells showing foci of polyoma virus-transformed cells 21 days after infection. The cultures were seeded 1×10^4 cells infected with 5×10^8 PFU of virus. (left) RLV-infected RE culture; (right) uninfected RE culture.

FA tests; (iii) reproducibility of the results.

Transformation of RE cells by polyoma virus has previously been reported (8, 9). Our data indicate also that transformation by polyoma virus was facilitated by the use of established RE cell lines instead of primary or secondary cultures, a finding previously reported in other systems (1, 10).

Although it has been shown that cells transformed by DNA or RNA tumor viruses are still susceptible to reinfection and "double" transformation by a second tumor virus (11–14), our current finding provides another example of enhanced susceptibility to transformation by a DNA virus of cells carrying a nontransforming RNA tumor virus. This enhancement could be due to an early event in virus–cell interactions (more efficient absorption, penetration, and uncoating) or due to intracellular changes induced by the leukemia virus producing cells which render them more susceptible to transformation by polyoma virus. Another possible explanation is based on the oncogene hypothesis of Huebner and associates (11–14) which postualtes that the transforming oncogene known to be present in the C-type RNA viruses and in normal mouse, hamster, and chicken cells is

TABLE I. Transformation Efficiency of Polyoma Virus[a] in Rauscher Leukemia Virus (RLV)-Infected and Uninfected Rat Embryo (RE) Cell Lines.[b]

RLV-infected RE cells (F-119)		Uninfected RE cells (F-111)	
No. of cells/plate	Av no. of transformed foci/plate	Av no. of transformed foci/plate	Ratio of transformation efficiency of RLV/RE: control/RE
1×10^5	380	16	22.5
5×10^4	231	8	28.9
1×10^4	125	6	20.8
5×10^3	90	3	30.0

[a] Polyoma virus titer = 5×10^8 PFU.

[b] These cell lines were obtained from Dr. A. E. Freeman.

also present in the rat cells as an endogenous C-type RNA virus genome, and that the transformations induced by polyoma virus in the normal cells and the accelerated transformation in the RLV-infected cells are determined by endogenous and injected C-type RNA oncogenes, respectively.

Summary. Transformation of the established cell lines of Rauscher leukemia virus (RLV)-infected and uninfected rat embryo cells by polyoma virus is reported. Transformation was evidenced by morphological changes and presence of polyoma "T" antigens by means of complement-fixation test and fluorescent antibody staining technique. Increased transformation efficiency of polyoma virus was observed in rat embryo cells infected with RLV.

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