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Isolation of Simian Virus 40 from a Newborn Child

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Rubella virus and simian virus 40 (SV40) were isolated from a newborn child suffering from neurological and anatomical anomalies. The SV40 isolate was very similar to SV40 strain 777 by electron microscopic, biological, and immunological criteria.

Several papovaviruses have been isolated from human adults during the past few years. One agent, the BK virus, was excreted in the urine of a patient who had undergone a renal transplant (1). Another virus was isolated from the brain of a patient suffering from progressive multifocal leukoencephalopathy (2). Simian virus 40 (SV40)-related antigens have been detected in some human meningiomas (7). Two further isolates from the brains of progressive multifocal leukoencephalopathy patients proved to be closely related to SV40 of monkey origin (3, 6). The isolation of SV40 has been reported from human melanoma metastases (5). The isolation from an infant of an agent exhibiting characteristics of SV40 is described here.

The infant, E.Lo., was the third child of Spanish guest workers in Germany. The first child died at the age of 2 days from an unknown disease, and the second child was suspected to have a congenital heart disease. At the birth of E.Lo., the mother was 27 and the father 35 years old. During the second month of pregnancy, the mother acquired rubella virus infection. The first sign of illness of E.Lo. was cyanosis some hours after birth (January 1974). Later, an open ductus Botalli, weak muscle tonus, reduced motoric action and other neurological anomalies, an elevated cerebrospinal fluid protein content, and signs of retardation became evident. The open ductus Botalli was ligated at the age of 5 months. The infant suffered from multiple feverish periods with enteritis during which it was fed by a gastric tube. The body weight of 3,160 g at birth increased only to 3,660 g 6 months later. With short intermissions the infant remained hospitalized permanently. With signs of hyperpyretic dehydration and thrombopenia, the infant died at the age of 15 months in a prolonged shock. Autopsy was not allowed by the parents.

Attempts to isolate a virus were made by inoculating cerebrospinal fluid, obtained at the age of 3 weeks, on primary African green monkey kidney (pAGMK) cells. After two passages, cytoplasmic vacuoles appeared as the first sign of cytopathic changes (Table 1). The agent could be grown to a titer of 10^8 mean tissue culture infective doses ($TCID_{50}$) on pAGMK cells. It proved to be ether resistant. The cytopathic effect did not develop in the presence of cytosine arabinoside (20 μg/ml).

Immunofluorescence assays of the infected pAGMK cells, performed with monkey antisera against SV40 (strain 777), revealed early protein and capsid antigen nuclear fluorescence typical for SV40. With human adenovirus antisera no cross-reaction was found. The agent was also isolated from throat swabs taken when the child was 13 and 14 weeks old. Isolation from throat specimens was also successful with TC7 cells, a CV1 subline of AGMK cells (Table 1). We could not detect antibodies in the patient's serum either by means of immunofluorescence tests against tumor (T) and capsid antigens of the agent and of SV40 (777), or by neutralization tests with both viruses. The sera was obtained when the child was 9, 15, and 60 weeks old.

Serum from the father (taken 2 years after the child's birth) exhibited no titer in the neutralization test with the agent and with SV40 (777) and showed a weak nuclear and cytoplasmic immunofluorescence at a 1:5 dilution with TC7 cells infected with the viruses. The mother's serum was negative in the same tests. Rubella virus was not detected in the specimens that were positive for the SV40-like agent. However, rubella virus was isolated from a throat swab taken when the child was 1 year old (Table 1). The child had rubella hemagglutination inhibition antibodies from birth on, reaching a titer of 1:512 (Table 1).

The agent from the infant, E.Lo., designated as SV40 (ELO), was compared with SV40

TABLE 1. *Results of virus isolation and serology*

Age of E.Lo. (weeks)	Specimen	Papovavirus ELO virus		Rubella virus	
		Isolation	Antibody[a]	Isolation	Antibody titer[b]
1	Serum				1:128
3	Cerebrospinal fluid	+[c]		–[d]	
4	Feces	–		–	
9	Serum		–		1:64
11	Serum				1:512
13	Throat swab	+[c]		–[d]	
14	Throat swab	+[e]		–[d]	
14	Urine	–			
15	Serum		–		
52	Throat swab	–		+	
52	Urine	–			
60	Serum		–		1:256

[a] Neutralization and immunofluorescence tests were used to detect antibodies.
[b] HAI, Hemagglutination inhibition antibodies.
[c] On primary AGMK cells.
[d] Presence of SV40 (ELO) hindered further testing for rubella virus.
[e] On primary and permanent (TC7) AGMK cells.

(strain 777) in terms of biological and antigenic criteria. The electron microscopic examination of a concentrated SV40 (ELO) virus preparation revealed typical papovavirus particles. When pAGMK cells were infected with SV40 (ELO), cytoplasmic vacuoles appeared after 2 to 3 days.

In cell cultures infected with SV40 (777) at the same multiplicity of infection, the vacuoles normally appeared with a delay of 24 h. The time course of nuclear fluorescence with SV40 (777) anti-T and capsid antigen sera was the same for SV40 (ELO) and SV40 (777) in pAGMK and TC7 cells. The appearance of the T-antigen fluorescence was the same for SV40 (777) and SV40 (ELO). However, the SV40 (ELO) capsid fluorescence at 24 h postinfection exhibited irregular nuclear patches but 24 h later was as homogenous as that in SV40 (777)-infected cells. These nuclear patches permitted differentiation between SV40 strains 777 and ELO.

In human skin fibroblast cultures, 1 to 5% of the cells reacted positively with SV40 (ELO) T antiserum 24 h after infection with 10^6 to 10^7 $TCID_{50}$ of SV40 (777) or SV40 (ELO). In monkey cells, cytosine arabinoside (20 and 40 μg/ml) inhibited the formation of SV40 (ELO) nuclear capsid antigen fluorescence as strongly as it did with SV40 (777).

In neutralization tests we found that both SV40 (777) and SV40 (ELO) (10^3 $TCID_{50}$) were neutralized by monkey anti-SV40 (777) serum to the same serum dilution (1:160). Immunofluorescence tests with serial dilutions of this serum exhibited the same end point of capsid immunofluorescence in pAGMK cells infected with SV40 (ELO) and SV40 (777).

The oncogenic potential of the agent was then examined by subcutaneous inoculation of 5×10^6 $TCID_{50}$ into newborn hamsters. Between 3 and 6 months later, 4 of 20 surviving animals developed tumors at the site of inoculation. These animals developed antibodies against SV40 (777) and (ELO) T antigen, as shown by immunofluorescence assays. From one tumor, a cell line was established. More than 95% of the cultured cells exhibited nuclear fluorescence with sera from hamsters with SV40 (777) and SV40 (ELO) tumors.

When SV40 (ELO) tumor tissue ($>10^5$ cells) was dispersed by collagenase and inoculated subcutaneously into adult hamsters, 5 of 10 animals developed tumors. In one case, two transplanted tumors (1.5 cm) first regressed almost completely and then reappeared. The histopathological examination of two of the primary tumors, kindly performed by W. Oehlert from the Department of Experimental Pathology, revealed sarcomas. Thus, SV40 (ELO) virus resembles the SV40 strain 777 in most of the parameters investigated so far.

The reliability of our virus isolation procedures seems to us to be guaranteed by the following facts: (i) cerebrospinal fluid and throat swabs were processed at different times with pAGMK cells of different animals. (ii) The throat-swab specimens were kept sealed and frozen. Six months after the first attempt, reisolation from the same material was successful with pAGMK cells and with the permanent

line, TC 7. During all procedures, we took special precautions to avoid any contamination. (iii) Rhesus monkey kidney cultures, which could harbor SV40 as a latent infection, were not used in the laboratory. (iv) SV40 was not used in the virus isolation building or by the staff working in that building. (v) Of several hundreds of African green monkey cell cultures used for virus isolation in our laboratories, the kidney cells were never found to be contaminated by SV40.

The biological significance of this isolation of a SV40-like virus (besides the rubella virus isolation) from a infant remains unknown. Dystrophy and death of the infant provide no evidence for an etiological role of the papovavirus.

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