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Further Studies of a Simian Virus 40-Like Virus Isolated from Human Brain

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A virus similar to simian virus 40 was reisolated from brain homogenates of a patient with progressive multifocal leukoencephalopathy onto cultures of human fetal brain cells.

During the past year, three papovaviruses have been isolated from brains of patients with progressive multifocal leukoencephalopathy (PML), a subacute human demyelinating disease. Padgett et al. (2) reported the isolation of a papovavirus which showed, by immunofluorescent staining, no relationship to polyoma, human papilloma, or simian virus 40 (SV40) viruses. We reported the isolation of two agents from patients with PML which are closely related antigenically to SV40 by neutralization and immunofluorescence studies (4). The Padgett agent was isolated by inoculation of homogenates of a patient's brain onto human fetal brain cultures (HFB). Our initial isolation involved growing primary dispersion cultures of two patients' brains, subculturing, and then fusing with primary African green monkey kidney (PAGMK) cells in the presence of inactivated Sendai virus. This method raised the question of possible contamination or genetic recombination with simian viruses in the monkey cells (1). For this reason reisolation directly in HFB was attempted.

Brain homogenates were prepared in a manner similar to that described by Padgett et al. A frozen brain specimen from a previously reported case (case 2) (4) was used. A 1.4-g block of white matter in which virions had been seen by electron microscopy was rapidly frozen and thawed three times, minced finely, and ground in a chilled mortar. A 10% homogenate was made in phosphate-buffered saline (*p*H 7.4) containing 1% sodium deoxycholate and 0.025% trypsin. The homogenate was stirred for 30 min at 37 C; 1.5-ml samples were layered onto 6 ml of 5% sucrose in 0.02 M tris(hydroxymethyl)aminomethane (Tris) buffer (*p*H 7.5) and centrifuged at 100,000 × *g* for 2 hr. Pellets were suspended on 0.001 M Tris buffer (*p*H 7.8) containing 0.01% bovine serum albumin; fraction 5 suspension was centrifuged at 1,000 × *g* for 30 min, and the supernatant fraction was stored at −70 C.

Brain homogenate (0.6 ml) was mixed with an equal volume of Earle's minimum essential media (MEM) and adsorbed for 3 hr on monolayers of HFB. HFB cultures from 7- to 10-week-old fetuses were initially prepared by dispersion methods described by Shein (3). Subsequently cultures were obtained with greater spongioblast and smaller fibroblast populations by removing meninges, choroid plexus, and visible blood vessels from brain, mincing tissue, and expelling through a 23-gauge needle into plastic flasks. The tissue was initially moistened with 2 ml of growth media (MEM with 10% fetal calf serum, L-glutamine, and antibiotics), and after 48 hr a further 10 ml of media was added. By using Shein's method, monolayers developed in 7 to 10 days and by explant method within 2 weeks. Cultures were maintained in MEM with 2% fetal calf serum.

Inoculated HFB cultures were subpassaged at 28-day intervals by freezing and thawing and clarification by centrifugation at 1,000 × *g*. Eighteen days after the third passage in HFB, cytopathic effects (CPE) developed consisting of pyknosis and lysis of cells (Fig. 1). Indirect fluorescent antibody staining of these cells with monkey anti-SV40 serum, rabbit anti-PML agent serum, and serum from a patient with PML (case 1 in reference 2) demonstrated nuclear fluorescence with relative sparing of the nucleolus (Fig. 2). Electron microscopy of HFB cells showed papovavirus-like particles morphologically indistinguishable from the SV40-polyoma group (Fig. 3).

The reisolated agent produced CPE on initial passage in PAGMK cells. After 7 days, small vacuoles developed in cytoplasm followed by cell lysis. Initial passages in BSC-1 cells failed to pro-

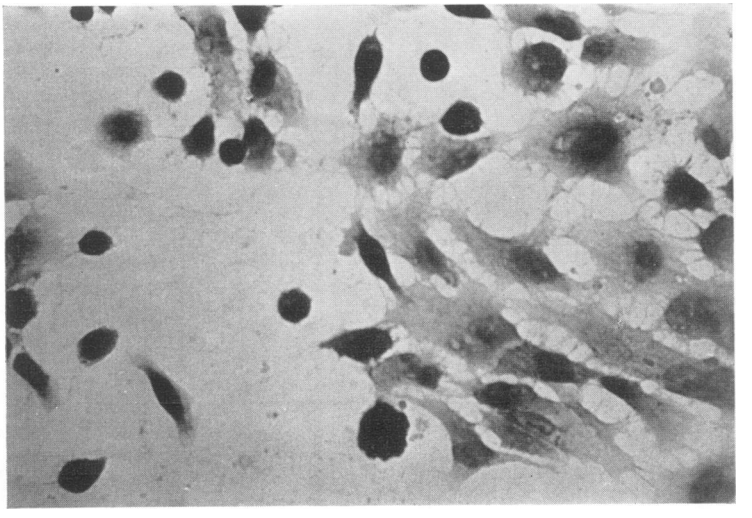

FIG. 1. *Focal pyknosis of HFB cells 18 days after inoculation with the second passage of brain homogenate. HE ×230.*

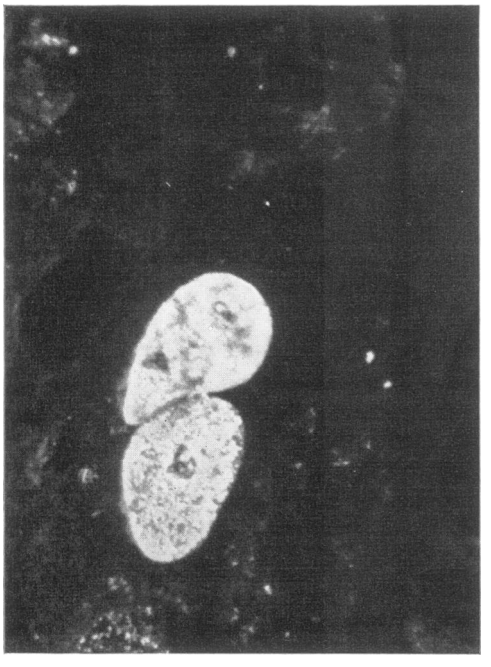

FIG. 2. *Nuclear fluorescence in spongioblast cells of human fetal brain culture inoculated with PML brain homogenate and stained with rabbit anti-SV40. ×750.*

duce CPE, but on third passage CPE was seen resembling that of SV40. Adaptation to BSC-1 cells was confirmed by fluorescent-antibody staining. Subsequently, virus was also isolated from the brain homogenate directly on PAGMK cells but only after four blind passages at 2-week intervals.

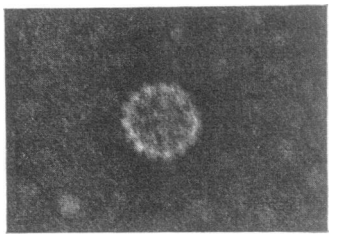

FIG. 3. *Electron micrograph of a single virion measuring 34 nm, negatively stained with 1% phosphotungstic acid. ×300,000.*

Neutralization of viruses derived from the HFB and PAGMK cultures demonstrated antigenic similarity as well as their relationship to SV40. Twofold dilutions of sera shown in Table 1 were incubated for 1 hr at 37 C with 100 median tissue culture infective doses of virus and assayed on PAGMK cells. Sera were similarly tested with polyoma virus and assayed in mouse embryo fibroblasts.

In summary, the SV40-like virus isolated directly from a homogenate of brain in HFB appears identical to that isolated in PAGMK fused to cultures derived from the patient's brain. This further confirms the human origin of the agent and indicates that the SV40 antigenic phenotype did not result from recombination of the human agent with possible simian agents in the PAGMK cultures. The growth of this agent

TABLE 1. *Neutralization tests*[a]

Sera	PML agent		SV40	Mouse polyoma
	HFB isolate	Previous PAGMK isolate-case 2		
Patient's serum-(case 1; 4-12-71)	1,280[b]	1,280	640	<10
Antiserum against case 2 virus (rabbit)	640	640	640	<10
Antiserum against SV40 (monkey)	640	320	640	<10
Antiserum against mouse polyoma (rabbit)	<10	<10	<10	160

[a] Abbreviations: PML, progressive multifocal leukoencephalopathy; HFB, human fetal brain; PAGMK, primary African green monkey kidney; SV40, simian virus 40.

[b] Titers expressed as reciprocal of final dilution neutralizing 100 median tissue culture infective dose of virus.

in PAGMK and BSC-1 cells provides further evidence for the similarity of the biological characteristics of this agent to known laboratory strains of SV40.

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