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# ISOLATION OF A SV40-LIKE VIRUS FROM A PATIENT WITH PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY

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*Summary.* — A SV40-like virus was isolated from the brain of a patient with progressive multifocal leukoencephalopathy. The virus was antigenically and serologically indistinguishable from SV40 wild type. A unique difference from SV40 was its ability to grow on human glial and CV-I monkey cells. The molecular weight of the viral DNA was very similar to that of SV40 DNA and the DNA cleavage patterns obtained after digestion with Hind II and Hind III restriction endonucleases were indistinguishable from those of SV40.

*Key words: human papovaviruses; SV40; progressive multifocal leukoencephalopathy*

## *Introduction*

Progressive multifocal leukoencephalopathy (PML) is a subacute demyelinating disease of man, usually occurring as a complication of a previous disorder of the reticuloendothelial system but it has also been reported in patients therapeutically immunosuppressed for other diseases or for organ transplantation (Weiner and Narayan, 1974). In the last years several reports appeared on the detection of papovavirus-like particles in and their isolation from the brains of patients with PML (JC, SV40 PML viruses) and from the urine of renal transplant patients (BK virus) (Padgett and Walker, 1976; Mäntyjärvi, 1979). Two of these isolates, JC and BK viruses, proved to be new human papovaviruses of the SV40/polyoma virus subgroup. Although they share some common properties and a set of DNA sequences with SV40 they are clearly different from the latter and from each other (Padgett and Walker, 1976; Law *et al.*, 1979). On the other hand, Weiner *et al.* (1972) isolated papovaviruses from brain tissues of two patients with PML (SV40 PML-1; SV40 PML-2); these viruses proved to be antigenically indistinguishable from each other and from SV40. A comparison of the DNA of one isolate (SV40 PML-2) with that of SV40 by digestion with Hind II/III restriction endonucleases revealed only minor differences confined to the hypervariable

regions of SV40 DNA (Sack *et al.*, 1973). However, a unique difference from SV40 was the ability of SV40 PML-2 to grow efficiently in human and monkey cells with cytopathic effects leading to complete cell lysis. SV40 is quite different in this respect and replicates in human cells very inefficiently (Martin *et al.*, 1974).

JC virus was isolated from or identified in more than 30 brain specimens of PML patients and several reports have shown a high percentage of seropositivity to JC (and BK) virus in sera from persons in remote geographical areas, indicating that JC and BK viruses are common human infectious agents (Law *et al.*, 1979; Brown *et al.*, 1975). On the other hand only two cases of SV40 PML mentioned above, were detected in the brains of PML patients and only low titres of antibodies to SV40 were found in human sera (Brown *et al.*, 1975; Padgett and Walker, 1976). A number of additional observations have been made concerning association between SV40-like viruses and human tumours. Isolates of SV40-like viruses were reported from human metastatic melanomas (Soriano *et al.*, 1974; Geissler *et al.*, 1980).

In the present paper we report on the isolation of a papovavirus from a PML patient and its partial characterization. This isolate in its antigenic and biological properties proved to be very similar to SV40 but clearly different from BK and JC viruses.

## *Materials and Methods*

*Brain material.* The autopsy material was obtained from a patient of the Institute of Pathology, Martin-Luther-University Halle/Saale (see Jänisch *et al.*, 1977). The frozen brain material was minced with scissors and small pieces of it were used in the present experiments. In some cases the material was homogenized in Eagle's minimum essential medium (MEM) using a Teflon homogenizer.

*Cell cultures.* The human glial cell line U787CG (HG) was obtained from Dr. B. Westermark (Uppsala). The cells were grown in Eagle's MEM supplemented with 10 % foetal calf serum (Flow Laboratories). The CV-I monkey cells were originally obtained from Dr. M. Girard (Villejuif). They were grown in Eagle's MEM supplemented with 10 % calf serum and non-essential amino acids. The plauqe test was performed on CV-I cells using a 20 mM Tes buffere system as described by Itagaki and Kimura (1974).

*Isolation of virus and viral DNA.* Human glial and CV-I monkey cells were inoculated with homogenates of brain material and the virus was propagated on the same cells. The viral DNA was extracted from infected human glial and CV-I monkey cells by the procedure of Hirst (1967) and purified as described by Böttger *et al.* (1976).

CV-I cells were infected with SV40 ($10^{-4}$ PFU/cell) and SV40 DNA was extracted and purified as described above.

*Characterization of viral DNAs.* Restriction endonuclease digestion and polyacrylamide gel electrophoresis were done as described by Scherneck *et al.* (1979*a*).

*Serological tests.* Neutralization and haemagglutination tests were performed as described by Scherneck *et al.* (1979*a*).

*Immune sera and immunofluorescence.* The sera were prepared as described (Scherneck *et al.*, 1979*b*) and all staining procedures were done by the indirect technique with fluorescein isothiocyanate-conjugated anti-globulins according to the method described by Scherneck *et al.* (1979*a*).

*Electron microscopy.* Cell cultures were centrifuged at $600 \times g$, whereupon the pellets were fixed with 2 % (w/v) glutaraldehyde in 0.05 M phosphate buffer pH 7.4 for 90 min at 4 °C and incubated overnight in phosphate buffer. Postfixation was performed with 2 % (w/v) osmium tetroxide for 2 hr and the cells were embedded in EPON 812. Sections were stained with an uranyl

acetate solution in 50 % ethanol (saturated at room temperature). Ultrathin sections were made with an KLB ultratome I, poststained with lead citrate and viewed under a JEM 100 B (Jeol) electron microscope. DNA was spread by a protein-free technique using carbon-coated grids charged in amylamine vapor. After adsorption of the DNA the grids were stained with 2 % (w/v) uranyl acetate, rotatory shadowed with platinum at an angle of 10° and examined with a JEM 100 B microscope.

## *Results*

### *Detection of papovavirus-like particles in the PML autopsy material*

Brain material was obtained from a 44 years old man who had been therapeutically immunosuppressed for renal transplantation. Six months after the transplantation he developed a disorder of the central nervous system and died 3 weeks later. Post mortem examination showed the characteristic morphological picture of PML. For details about the clinical course and histological data see Jänisch *et al.* (1977).

Examination of the autopsy material in the electron microscope revealed, in analogy to the results published by Jänisch *et al.* (1977), papovavirus-like particles within a few nuclei of oligodendrocytes. On the other hand the enlarged, morphologically abnormal astrocytes, a great number of which were found in the areas of demyelination, were free of virus. The virus particles were indistinguishable in size and morphology from virions of the SV40-polyoma virus subgroup (Fig. 1). In some cases a paracrystalline arrangement of the virions within the nuclei could be observed.

### *Growth of the PML virus in human glial and CV-1 monkey cells*

Both cell lines were inoculated with brain homogenates prepared directly from the autopsy material. Cytopathic effects (CPE) were not observed in either cell line until 12—18 days after infection (p.i.), but some of the cells remained undamaged and the CPE was not complete.

HG and CV-1 monkey cells were tested for the presence of SV-40-related T- and V-antigen by indirect immunofluorescence. About 10 % of both CV-1 and HG cells were positive for T-antigen and about 5 % were positive for V-antigen, when tested 15 days p.i. with the autopsy material. The percentage of cells positive for both antigens increased when the virus was subpassaged in the same cell lines. By subpassage 5 about 50—60 % of HG cells and 30—40 % of CV-1 cells were positive for T-antigen, while 30—40 % and about 25 % of HG and CV-1 cells, respectively, contained SV40-related V-antigen (Table 1). Although the virus was propagated in both cell lines the HG cells seemed to be a more favourable system. Attempts to infect CV-1 cells with virus originally grown in HG cells did not increase the virus yield on CV-1 cells, even after 5 subpassages (results not shown). With plaque purified virus, titres not higher than those menitoned above were obtained in both cell lines. Possibly the relatively small amounts of T- and V-antigen-positive CV-1 and HG cells, respectively, could be increased if primary cell cultures were used.

Electron microscopic examination of HG and CV-1 cells infected with the PML virus revealed papovavirus-like particles in numerous nuclei and in

**Table 1. Detection of SV40-like T- and V-antigen in HG and CV-I cells after infection with PML virus**

| Cells | % of cells positive for T-antigen | % of cells positive for V-antigen | PFU/ml |
|---|---|---|---|
| HG cells infected with the original brain material | 10 | 5 | $10^2-10^3$ |
| HG, subpassage 2 | 30–40 | 10 | Not tested |
| HG, subpassage 5 | 50–60 | 30–40 | $5 \times 10^4$ |
| CV-I cells infected with the original brain material | 10 | 5 | $10^2-10^3$ |
| CV-I, subpassage 2 | 20–30 | 10 | Not tested |
| CV-I, subpassage 5 | 30–40 | 15–20 | $5-8 \times 10^3$ |

the cytoplasm of both cell lines (Fig. 2). In both cell lines, tubular virus structures could occassionally be seen and sometimes 2 or more tubules formed bundles (Fig. 3; Geissler *et al.*, 1980).

*Haemagglutination*

JC and BK viruses agglutinate human group 0 erythrocytes whereas virions of SV40, SV40 PML and SV40 GBM do not so (Takemoto and Mullarkey, 1973; Weiner and Narayan, 1974; Scherneck *et al.*, 1979*a*). Haemagglutination tests were performed to test the degree of relatedness of our virus isolate to SV40. Virus suspensions or cells infected with the virus did not agglutinate human group 0 or guinea pig erythrocytes to a significant titre. No haemagglutination was observed at 4 °C or room temperature.

*Neutralization*

SV40 rabbit antiserum neutralized in our experimental procedure CV-I-propagated SV40 by more than 2 log units and the PML virus isolate grown in the same cells or in HG cells in each case cells from the 5th subpassage, by nearly 2 log units.

*Nucleic acid*

The viral nucleic acid extracted from virus-infected HG cells consisted of double-stranded super-coiled molecules (form I) and relaxed circles (form II). In CsCl-ethidium bromide isopycnic gradients the DNA banded at the same position as SV40 DNA. Electrophoretic examination showed that the viral DNA was apparently homogeneous and indistinguihable from SV40 DNA. However, estimation by electron microscopy showed that the molecular weight of the PML virus DNA was about 10–15 % higher than that of SV40 DNA (Fig. 4).

*Cleavage of the PML-DNA with restriction endonucleases*

A detailed analysis of the viral DNA was performed by means of site--specific restriction endonucleases, namely HindII and HindIII. The digestion products were fractionated by electrophoresis on 4 % polyacrylamide slab

gels. As shown in Fig. 5, the fragment patterns of SV40 and the PML virus produced after digestion with these two enzymes were very similar. In further experiments the PML virus DNA was digested with the EcoRI and BamH1 restriction enzymes. The cleavage patterns did not differ from that of SV40 (results not shown). In this respect our PML virus isolate was not only indistinguishable from SV 40, but also from SV40 PML-1 (Nathans, 1976) and only slightly different from SV40 PML-2 and SV40 GBM (Sack *et al.*, 1973; Scherneck *et al.*, 1979a). On the other hand the cleavage patterns of JC and BK viruses are clearly different from each other and from SV40 (Martin *et al.*, 1979; Howley *et al.*, 1975). Because of the limited amount of DNA, a more detailed restriction analysis of the PML-DNA was impossible.

## *Discussion*

A viral aetiology of PML has been proposed by several authors (Weiner and Narayan, 1974; Padgett and Walker, 1976; Padgett *et al.*, 1976). At least 3 different papovaviruses might be implicated as aetiologic agents in PML. Two of them (JC, SV40-PML) were isolated from the brains of PML patients, whereas the BK virus was detected in urine of a renal autograft recipient (Padgett *et al.*, 1976).

There are clear-cut differences in antigenic, serological and biological properties between JC and BK viruses on the one hand and SV40 and SV40 PML on the other (Padgett and Walker, 1976; Law *et al.*, 1979; Martin *et al.*, 1979). Tumours were induced in Syrian hamsters by JC virus, BK virus, SV40 and SV40 PML (Padgett and Walker, 1976). It is particularly notable that the original JC virus is extremely neurooncogenic; the tumours induced in hamsters include medulloblastomas, neuroblastomas, glioblastomas and meningiomas (Padgett *et al.*, 1977). Moreover, JC virus has been shown to induce brain tumours resembling human glioblastoma multiforme in nonimmunosuppressed owl monkeys (London *et al.*, 1978). In contrast, BK virus and SV40 PML induce tumours of the ventricular surfaces identified as choroid plexus papillomas or ependymomas (Becker *et al.*, 1976).

JC virus is most commonly associated with PML, but only 2 cases of SV40 PML have been identified in the brains of PML patients.

We isolated a virus from the brain autopsy material of a 44 years old PML patient which was morphologically, antigenically and serologically indistinguishable from SV40 wild type. The virus isolate could be propagated in CV-I monkey cells and continuous human glial cells. In this respect it resembles the SV40 PML viruses isolated by Weiner *et al.* (1972) and differs from the SV40 wild type. But no efficient virus growth was reached even after 5 subpassages in either CV-I or human glial cells. The low virus yields could be the consequence of inadequate cell culture systems for virus propagation because other human papovaviruses like SV40 PML grow efficiently in primary monkey (AGMK) and human glial (PHFG) cells (Weiner and Narayan, 1974). Another explanation of the low virus yields could be the presence of a large number of defective interfering particles

in the virus stock but the homogeneity of the viral DNA prepared from infected CV-I and HG cells does not support this assumption.

The electrophoretic mobility and the cleavage pattern of our virus DNA after digestion with HindII, HindIII, EcoRI and BamH1 restriction enzymes did not differ significantly from that of SV40 DNA. Therefore we can characterize it as a SV40-like virus which resembles in some respect (host range) the SV40-PML-1 virus. We could not confirm the longer clinical survival of the 2 cases of PML associated with the PML-1 and PML-2 viruses, respectively (Weiner *et al.*, 1972).

Serological studies indicate that JC virus, BK virus and to a lesser degree SV40 produce widespread subclinical human infections (Brown *et al.*, 1975).

Although the pathogenesis of PML is uncertain, there is some evidence that the foci of demyelination are a direct result of viral infection. The oligodendrocytes, the cells which maintain the myelin sheats, are fully packed with virus particles which appears to be the result of a lytic infection. Astrocytes, the other major glial cells of the white matter, only rarely contain virus particles, but they are distorted with bizarre nuclear forms and can contain JC (or other papovavirus) DNA copies (Dörries *et al.*, 1979). In this respect the astrocytes behave like virus-transformed cells. The hypothesis that oligodendrocytes are lytically infected and astrocytes acquire some characteristics of transformed cells is supported by in vitro findings (Shein, 1967). In PHFG cell cultures inoculated with SV40 or SV40-PML viruses, the spongioblasts, though to be precursors of the oligodendrocytes, were lysed but some astrocytes became transformed. In this connection it is interesting that 2 patients with PML exhibited multiple gliomas in the areas of PML (Richardson, 1961; Castaigne *et al.*, 1974;). The multiplicity of the gliomas in these cases of PML is intriguing in view of the findings that:

— JC virus induces gliomas and glioblastomas in newborn hamsters and owl monkeys (Padgett *et al.*, 1977; London *et al.*, 1978);

— a SV40-like virus, SV40-GBM, was isolated from a human glioblastoma multiforme (Scherneck *et al.*, 1979a; Geissler *et al.*, 1980);

— SV40-related DNA sequences have been found in a human glioblastoma (Smith *et al.*, 1977).

Moreover, a human glioblastoma cell line has been found to support lytic infection of SV40 wild type (O'Neill, 1976). The presence of JC virus DNA in vascular endothelial cells of brain sections of a PML patient was confirmed by in situ hybridization with JC virus cRNA (Dörries *et al.*, 1979). These findings should contribute to a better understanding of the papovavirus subclinical infection route and the pathogenesis of PML and human tumours in which SV40-like viruses have been detected (Soriano *et al.*, 1974; Scherneck *et al.*, 1979*a*; Geissler *et al.*, 1980; Takemoto, pers. comm.). But further studies in this field and especially the examination of large quantities of human tumours and brain material obtained from PML patients will be necessary for a better understanding of the evolution of SV40 in man.

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*Explanation of Figures* (Plates XXX—XXXIV):

*Fig. 1.* Electron microscopic demonstration of papovavirus-like particles in the original PML autopsy material. × 135 000.

*Figs 2 and 3.* Electron microscopic demonstration of papovavirus-like particles in CV-I monkey cells infected with the PML virus. *2* — Virions in the nucleus; × 182 000; *3* — Part of a bundle containing 2 tubules filled with PML virus; × 96 000.

*Fig. 4.* Electron microscopic demonstration of a PML virus DNA molecule. × 200 000.

*Fig. 5.* Comparison of SV40 (1, 2) and PML (3, 4) virus DNA fragments produced with HindII (1, 3) and HindIII (2, 4) restriction endonucleases by 4 % acrylamide slab gel electrophoresis followed by staining with ethidium bromide.