JOURNAL OF VIROLOGY, Apr. 1979, p. 384-389
0022-538X/79/04-0384/06$02.00/0

Vol. 30, No. 1

NOTES

JC Human Papovavirus Replication in Human Amnion Cells

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Received for publication 22 November 1978

JC human papovavirus was found to replicate in primary human amnion cells. The virus has undergone eight passages in amnion cells and was identified by serological methods as JC virus. By restriction endonuclease analysis of the viral DNA, the fragments observed were identical to those previously reported for the prototype strain.

JC virus (JCV), a human papovavirus, was first isolated from the brain tissue of a patient with progressive multifocal leukoencephalopathy (8). The virus was originally propagated in human fetal brain cells which, to date, have been the only cells capable of supporting JCV replication. Padgett et al. (9) tested a large number of different types of cells from various species for susceptibility to JCV with negative results. Because of this host restriction and the limited availability of fetal glial tissue, investigation of this interesting virus has been limited. Furthermore, fetal brain cell cultures suitable for JCV propagation must be rich in spongioblasts for high virus yields (9). Because of these difficulties, we have screened a number of cells for susceptibility to JCV in an attempt to develop alternative cell culture systems for growth of the virus. Among the many cells tested were primary human amnion cells (HEM Research, Inc., Rockville, Md.), which we have found suitable for the support of JCV growth to a limited extent.

Primary human amnion cells grown in 75-cm^2 plastic flasks were infected with 5 ml of JCV with a hemagglutination titer of 1:50. Amnion cells grown on cover slips were also infected for assay for viral (V) and tumor (T) antigens by the indirect fluorescent-antibody technique. After a 1-h adsorption at 37°C, Eagle medium containing 5% fetal bovine serum and antibiotics was added to the cultures.

The results of fluorescent-antibody studies on JCV-infected amnion cells are shown in Table 1. A few cells were positive for T-antigen on day 3 after infection when the cells were still negative for V-antigen. By day 5, 10% of the cells were synthesizing T-antigen, and a small percentage (0.3%) stained for V-antigen. The percentage of cells positive for both antigens increased with time, and by 14 days many of the cells were positive for T- or V-antigen. Many of the antigen-positive cells were greatly enlarged with swollen nuclei (Fig. 1). By 19 days, when 75% of the cells were positive for T-antigen, 30% contained detectable V-antigen.

Cytopathic effects were not observed until 10 to 14 days after infection, but the changes were subtle and difficult to detect. These changes consisted of scattered, round, highly refractile cells and bizarre-shaped cells (Fig. 2, top). These bizarre-shaped cells became detached and floated in the medium. Another characteristic change observed in infected cultures was the presence of giant cells measuring approximately five and six times the size of uninfected cells with greatly enlarged nuclei (Fig. 2, bottom). These large cells were positive for T- and V-antigens, as noted by the fluorescent-antibody technique in Fig. 1. Most of the cells remained healthy, and cytopathic effects were never complete.

The medium was changed weekly, and the floating, infected cells were collected by centrif-

TABLE 1. *JCV T- and V-antigen synthesis in human amnion cells*[a]

Day	% T-antigen positive	% V-antigen positive
3	0.05	Negative
5	10	0.3
8	40	20
14	65	25
19	75	30

[a] Amnion cells grown on cover slips were infected with 50 hemagglutination units of JCV. At various times after infection, cover slips were stained with JCV hamster anti-T- and rabbit anti-V-sera.

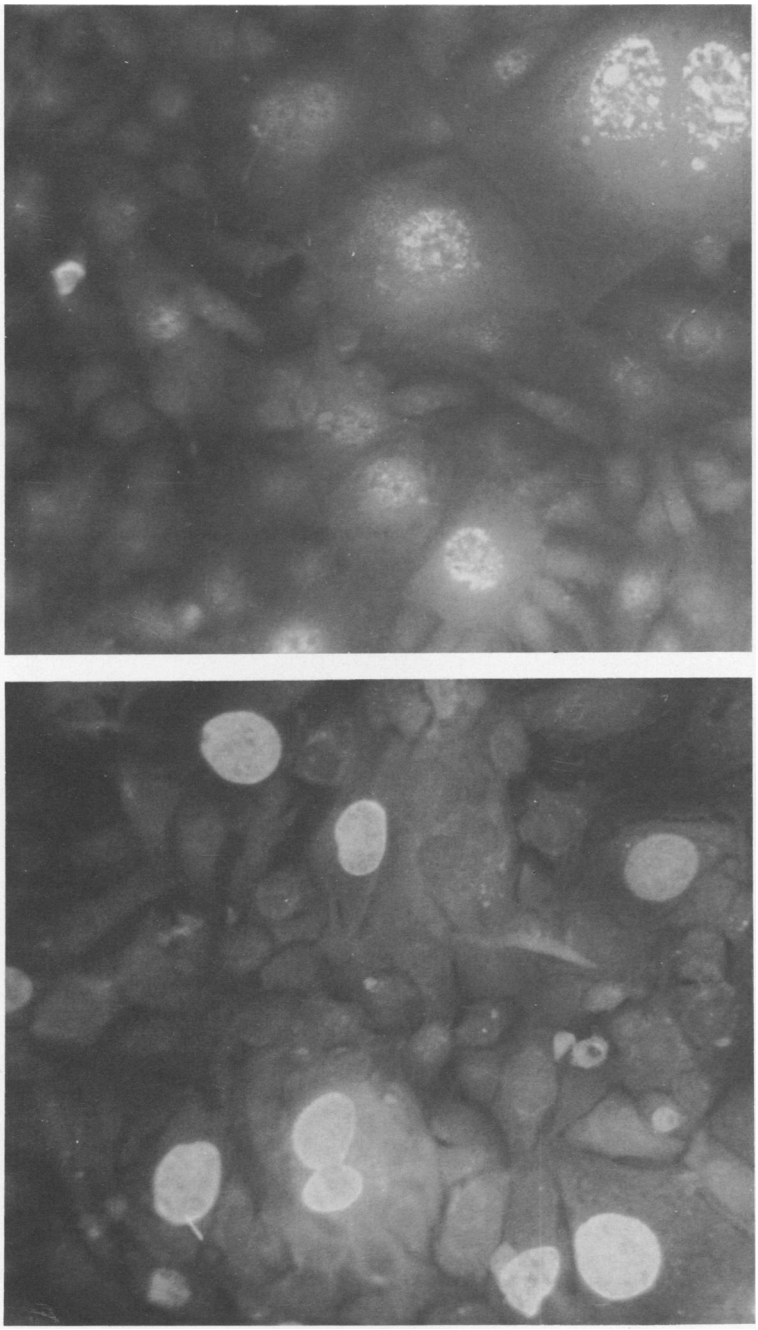

FIG. 1. *Immunofluorescence of JCV-infected human amnion cells at 10 days after infection. (Top) T-antigen; (bottom) V-antigen. ×250.*

ugation at 3,000 rpm. A portion of the pelleted cells was fixed in 2% glutaraldehyde and examined by electron microscopy. Many of the cells contained numerous, typical papovavirus virions within their nuclei (Fig. 3). Virus was extracted from pelleted cells by brief sonic treatment (Bronwill Sonifier) to disrupt the cells. Receptor-destroying enzyme (Microbiological Associates, Bethesda, Md.) was added to the disrupted cells at a concentration of 100 U/ml, and the

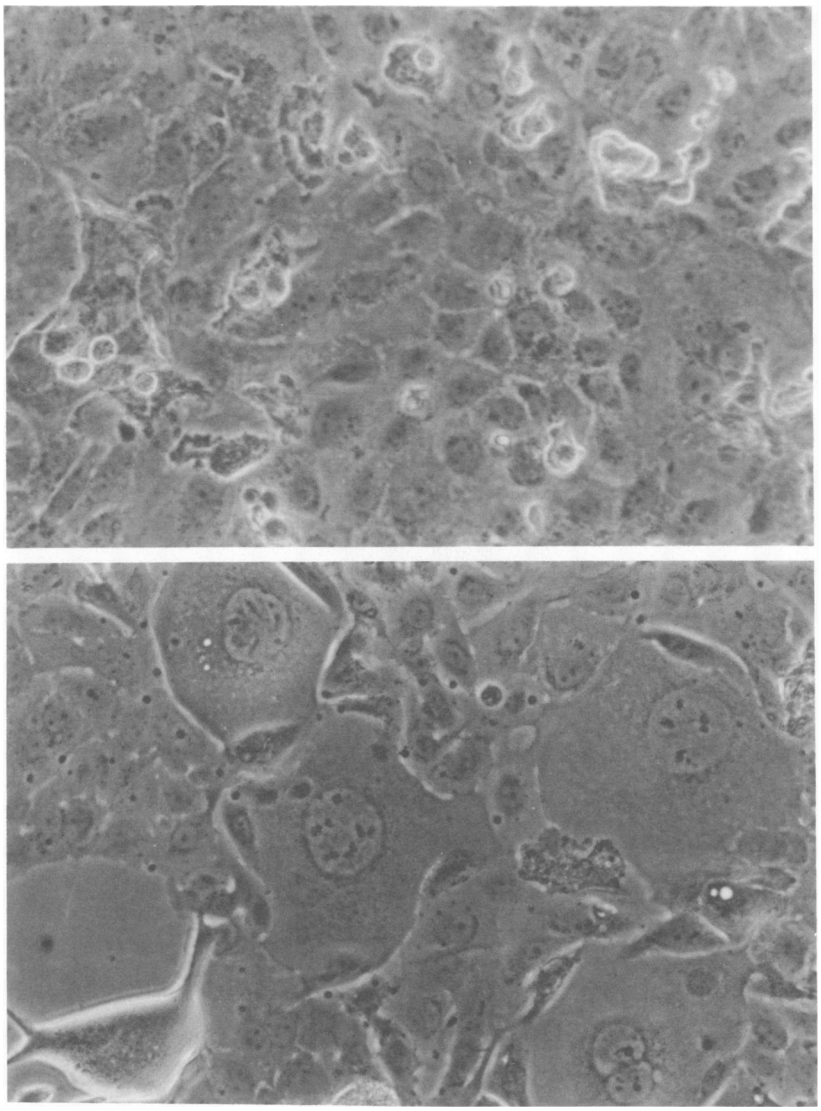

FIG. 2. *Cytopathic changes in human amnion cells infected for 2 weeks with JCV. (Top) Bizarre-shaped cells; (bottom) huge, giant cells.* ×250.

mixture was incubated at 37°C overnight. The virus-containing suspension was then clarified by centrifugation at 5,000 rpm, and the supernatant was tested for hemagglutinin, using human type O erythrocytes. The hemagglutination titer was 1:320, and the hemagglutinin was specifically inhibited by JCV antiserum.

To further characterize the JCV which had been grown in amnion cells, we examined the viral DNA synthesized. Radiolabeled viral DNA was purified directly from cells maintained in the presence of [*methyl*-^{3}H]thymidine (30 μCi/ml) at 12 days after infection by differential salt precipitation (2). The supercoiled, form I DNA with a density of 1.60 g/cm^3 was separated from linear DNA molecules by isopycnic centrifugation in a CsCl-ethidium bromide gradient (3). The supercoiled JCV DNA was further purified by sedimentation in a 5 to 30% neutral sucrose gradient as previously described (3).

The ^{3}H-labeled form I JCV DNA purified from infected amnion cells had a sedimentation coefficient of 19S (Fig. 4). The relationship between the sedimentation coefficient (S_{sc}) and the molecular weight (M) of supercoiled papovavirus DNAs has been investigated by Martin et al. (5) and can be expressed by the following equation: $S_{sc} = 0.0605\ M^{0.387}$

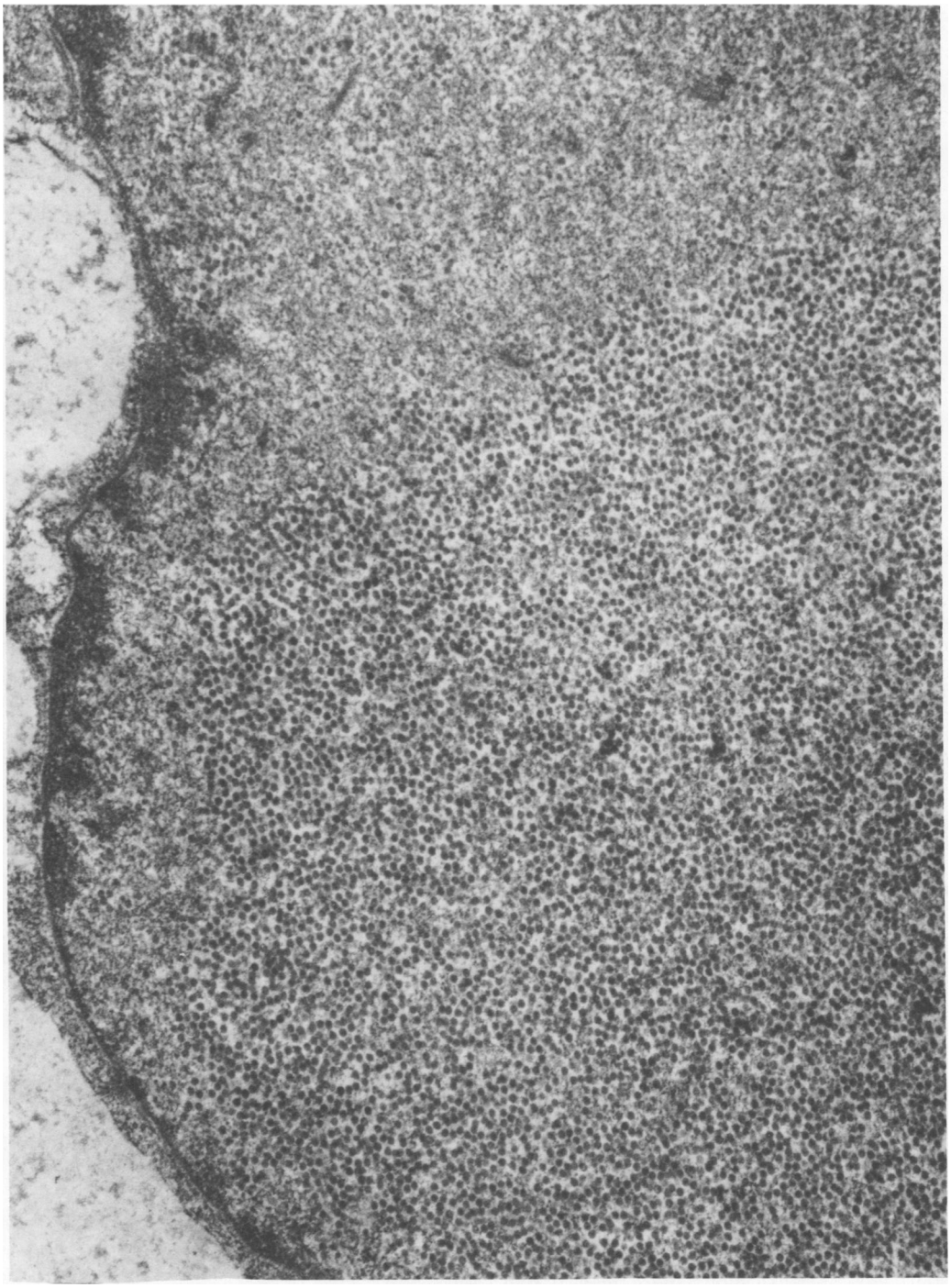

FIG. 3. *Electron micrograph of a portion of the nucleus of a JCV-infected cell showing numerous papovavirus particles. ×30,780.*

From the sedimentation coefficient of 19S, we can therefore calculate that the predominant species of supercoiled JCV DNA purified from amnion cells is 2.9×10^6 daltons. This value is in good agreement with the previous estimate of 2.93×10^6 daltons, based on the electron micro-

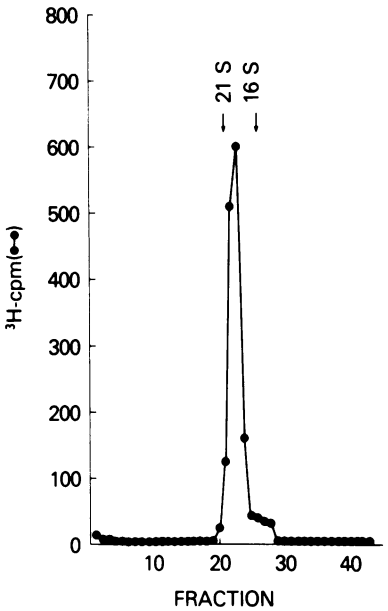

FIG. 4. *Sedimentation of purified JCV DNA in neutral sucrose. ^{3}H-labeled JCV DNA, purified from infected amnion cells, was mixed with ^{32}P-labeled simian virus 40 DNAs I and II and sedimented through a 5 to 30% (wt/vol) neutral sucrose gradient for 16 h at 10°C in a Beckman SW41 rotor. Fractions were collected from the bottom of the tube, and the radioactivity was determined by counting in Aquasol. The positions of simian virus 40 I (21S) and II (16S) are indicated by the arrows.*

scopic analysis reported by Osborn et al. (7).

The purified JCV DNA from amnion cells was further evaluated with restriction endonucleases and shown to be similar to JCV DNA obtained from infected HFB cells. Cleavage of the JCV DNA derived from amnion cells with either *Hpa*I or *Eco*RI (Fig. 5c and d) generates full-length linear molecules, indicating that the DNA contains one recognition site for each of these enzymes, in agreement with previous observations (7). Cleavage with either *Hin*dIII or *Hin*dII + III resulted in a heterogeneous, nonequimolar mixture of fragments, apparently due to the presence of defective molecules (Fig. 5a and b). These patterns are indistinguishable from those obtained by us from JCV-infected HFB cells, and the *Hin*dII + III pattern (Fig. 5a) is quite similar to that reported by Osborn et al. (7). Osborn et al. (6) have shown more recently that, with different isolates of JCV and with more homogeneous stocks of prototype JCV, cleavage with *Hin*dIII results in three fragments with molecular weights approximately 88, 8, and 4% of the simian virus 40 genome. These three bands are prominent in the *Hin*dIII cleavage of JCV DNA from amnion cells. Therefore, the DNA synthesized in amnion cells is JCV DNA, and the heterogeneity detected is presumably due to defective particles in our stock JCV.

JCV has undergone eight passages in amnion cells up to the present time. There is no apparent "adaptation" of the virus to growth in these cells since the development of cytopathic effects is still slow (10 to 14 days) and is never complete. An explanation for the low virus yields may be the presence of a large number of defective interfering particles in the virus stock. The heterogeneity of the viral DNA prepared from in-

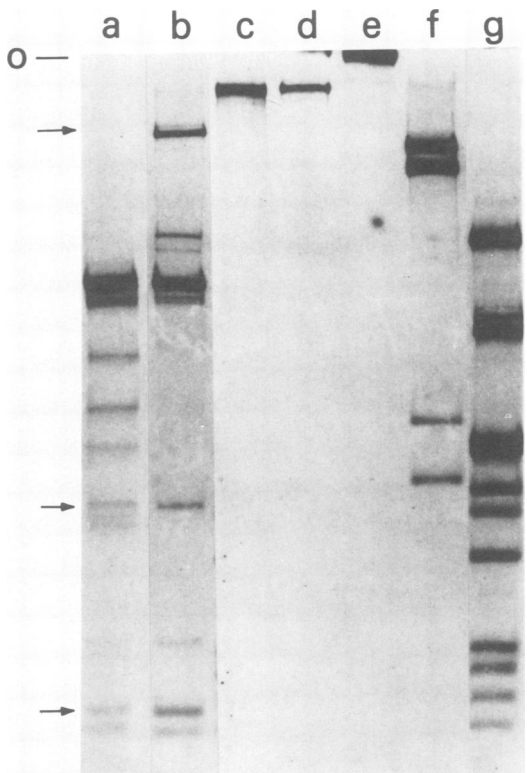

FIG. 5. *Restriction endonuclease cleavage products of JCV DNA purified from infected human amnion cells. Samples (50 μl) containing 4,000 cpm of (a) HindII + III digestion of JCV DNA, (b) HindIII digestion of JCV DNA, (c) HpaI digestion of JCV DNA, (d) EcoRI digestion of JCV DNA, (e) uncleaved JCV DNA, (f) HindIII digestion of BKV DNA, and (g) HindII + III digestion of simian virus 40 DNA were analyzed by electrophoresis in a composite 3% acrylamide-0.5% agarose slab gel as previously described (4). The gel was exposed for 4 weeks directly to Kodak X-ray film (XR-2) at −70°C after dehydration in dimethyl sulfoxide, impregnation with 2,5-diphenyloxazole, and drying (1). The arrows in the left-hand margin indicate the positions of the three JCV HindIII DNA fragments (b) seen in homogeneous stocks of prototype JCV (6).*

fected cells (data not shown) suggests that defective particles may indeed by present in large numbers. Furthermore, the studies on the time course of T- and V-antigen synthesis in infected cells showed that the percentage of T-antigen-positive cells was always substantially higher than the number of V-antigen-positive cells, an observation previously made by Padgett et al. (8) in JCV-infected human fetal glial cells. This would suggest that T-antigen-inducing defective particles are present in the original stock of JCV. Despite these problems, sufficient numbers of amnion cells can be infected to provide infectious virus, as well as viral DNA, for further biological and biochemical studies.

We thank C. F. T. Mattern and W. Daniel for the electron micrograph and Susan Hostler for her secretarial assistance.

T.M. is a Visiting Fellow, National Institute of Health, Tokyo, Japan.

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