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NOTES

Propagation and Primary Isolation of Papovavirus JC in Epithelial Cells Derived from Human Urine

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Human papovavirus JC, previously passaged in amnion cells or in primary human fetal glial cells, replicated efficiently in urine-derived epithelial cells. Primary isolation of the virus from brain extracts was possible in urine-derived cells, but these cells were not as sensitive as primary human fetal glial cells for this purpose. Primary isolations of human papovavirus JC from urine sediments of renal transplant patients were made in urine-derived cells.

JC virus (JCV), a human papovavirus, is the etiological agent of progressive multifocal leukoencephalopathy (PML), a demyelinating disease of the central nervous system. Although PML is a rare disease, infection with JCV is common. Sero-epidemiological studies indicate that approximately 70% of adults have JCV-specific antibodies (7). Aside from the central nervous system, the urinary tract is the only other known site of JCV replication. JCV is a slowly multiplying virus and has a restricted host cell range in vitro. The virus grows only in cells of human origin: fetal glial (6), embryonic kidney (4), amnion (10), endothelial (2), and adult brain (11). However, not all of these cells support efficient virus multiplication, and primary isolations have been made only in primary human fetal glial (PHFG) cells. We report here that urine-derived (UD) cells of epithelial morphology can support efficient replication of JCV and that these cells may be useful for primary isolation of this virus.

UD cells were cultivated from urine of uncircumcised male infants less than 2 weeks old by the method described by Felix et al. (3). Individual urine samples, in volumes of 1 to 20 ml, were centrifuged at 250 × *g* for 5 min at room temperature. Pelleted cells were suspended, without counting viable cells, in 5 ml of Eagle minimum essential medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with L-glutamine, nonessential amino acids, and containing 15% fetal bovine serum. Cell suspensions were seeded into 25-cm^2 cell culture flasks and incubated at 37°C in an atmosphere of 5% CO_2. Colonies of viable cells were first observed 4 to 7 days after seeding of cultures. A total of 23 of 68 urine specimens tested (34%) yielded viable cultures and could be maintained for 2 to 11 passages. Twenty-two specimens were discarded within the first 4 days of incubation due to microbial contamination. Twenty-three specimens did not grow, with growth being defined as establishment of a confluent culture which could be subcultivated at least once. Confluent cultures were passed with a 0.25% trypsin–0.02% EDTA solution (GIBCO) at a split ratio of 1:4 or 1:5. The total number of cells obtained from one specimen after four to five subcultivations ranged from 10^7 to 10^9. On average, cells grown successfully from a single urine specimen could be passed four times with a total yield of 6×10^7 to 7×10^7 cells. Our results are similar to those described in more detail by Felix et al. (3).

The ability of UD cells to support JCV replication was tested by inoculation of cell culture-adapted JCV. The virus preparations tested were the 14th passage of amnion-adapted JCV (10) and PHFG-adapted JCV MAD-1 (3rd and 6th passage) and JCV MAD-14 (2nd passage). The first passage of amnion-adapted JCV in UD cells produced a cytopathic effect (CPE) which was apparent at 7 days postinoculation (p.i.). Early cellular changes consisted of foci of greatly enlarged cells which contained swollen and, occasionally, multiple nuclei (Fig. 1). By 11 to 14 days p.i., infected cells began to detach from the monolayer and could be seen floating in the culture fluid. By 21 to 27 days p.i., large areas of the monolayer were detached, and the remaining

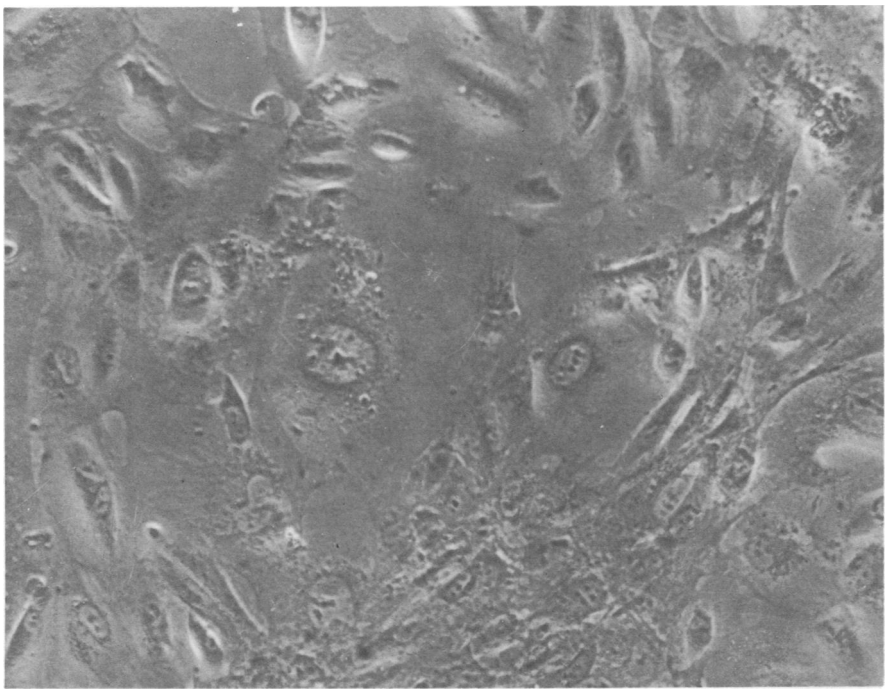

FIG. 1. Early cytopathic changes in JCV-infected UD cells (×390).

attached cells had become necrotic with accumulation of granular debris and degeneration of nuclear membranes. JCV was readily adapted to growth in UD cells. By the fourth passage, almost all cells in inoculated cultures became infected in 2 to 3 weeks, as determined by indirect fluorescent antibody staining (9) of cells grown on cover slips (Table 1). Indirect fluorescent antibody staining demonstrated that the greatly enlarged nuclei were positive for JCV capsid (V) antigen and that cells which appeared morphologically normal also expressed this antigen (Fig. 2). UD cells from different infants and at different passage levels were uniformly susceptible to JCV infection as judged by the time of appearance of V antigen after inoculation of a standard amount of UD cell-passaged JCV.

Viral hemagglutinin (HA) extracted from infected cells was detected by HA tests employing human type O erythrocytes. Viral HA was highly cell associated, and treatment of infected cells with receptor-destroying enzyme (Microbiological Associates, Bethesda, Md.) (10) increased HA titers 32- to 64-fold. When UD cells grown to confluence in 25-cm^2 flasks were inoculated with 32 HA units of UD cell culture-adapted JCV, total virus yields of 3×10^4 to 8×10^5 HA units were obtained after repeated harvesting and pooling of infected cells.

Three preparations of PHFG cell-adapted JCV were examined for infectivity in PHFG and UD cells. Cells were harvested at 14 days p.i. and stained by the indirect fluorescent antibody method for JCV V antigen. All three virus preparations were positive at 14 days p.i. in both PHFG and UD cells. The number of infected cells was 10-fold less in UD cells than in PHFG cells, but serial passage in UD cells resulted in adaptation of the viruses to this system.

To evaluate the usefulness of UD cells for primary isolation of JCV from brain tissue, parallel experiments were performed with PHFG and UD cells. Human brain extracts were prepared from autopsy material of five histologically confirmed cases of PML and from one

TABLE 1. JCV V antigen synthesis in UD cells inoculated with amnion-adapted virus

Day p.i.	% Of cells positive for antigen[a]		
	Passage 1	Passage 2	Passage 4
4	0.05	0.1	9
7	5	20	49
11	20	50	64
14	25	62	80
21	85	95	99

[a] The percentage of cells positive for antigen was determined by a count of antigen-containing cells divided by all cells in five areas of a cover slip. Inoculum for first and second passage, 5120 H,A units; fourth passage, 512 HA units.

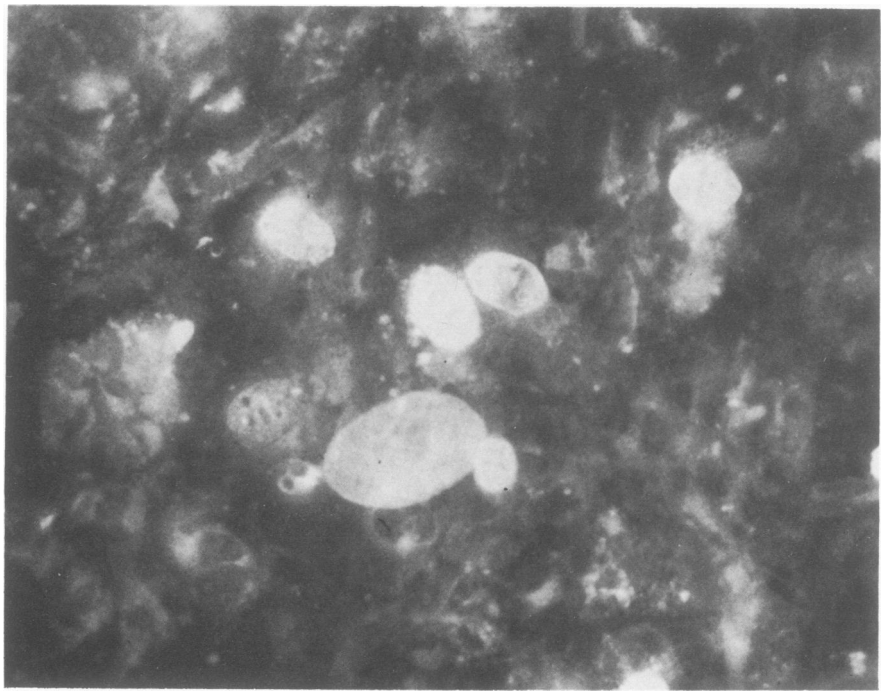

FIG. 2. Immunofluorescence of JCV-infected UD cells, V antigen (×660).

normal brain (5) and were inoculated onto PHFG and UD cells. Cells were harvested at various times p.i. and stained for V antigen (Table 2). JCV could be isolated from PML brain material in UD cells, but these cells were not as sensitive as PHFG cells for this purpose. Primary isolation of JCV in UD cells required prolonged incubation or blind passage before V antigen could be detected. Isolate no. 1 has been passed in UD cells and has become adapted to these cells, inducing more rapidly progressing CPE with greater production of virus.

Twenty-one urine specimens from 11 renal transplant patients who showed serological evidence of JCV infection were tested for infectivity on UD cells. The urines were collected and stored frozen in the first 6 months after transplantation, the time span in which antibody rise to JCV was detected (C. Andrews and M. Hirsch, personal communication), and were kindly supplied by M. Hirsch. JCV was isolated from three specimens from three different patients. Virus was first detected in the infected cultures 3 to 8 weeks p.i. by demonstration of V antigen or by observation of viral CPE (Table 2). Sack et al. (8) have reported that BK virus also replicates in UD cells, and we have successfully used this culture system for isolation of BK virus from urine specimens of several persons (A. M. Beckmann and K. V. Shah *in Polyoma Viruses and Human Neurological Disease*, in press).

Using PHFG cells, one of us (B.P.) has had only limited success in isolating JCV or BK virus from frozen urines known to contain papovavirus inclusion-bearing cells. The ease with which virus isolations were made in UD cells suggests that these cells may be more suitable than PHFG cells for primary isolation of virus from urine. However, this possibility could not be directly investigated because the quantities of

TABLE 2. Time of appearance of JCV antigen or CPE in UD cells after inoculation of PML brain materials and urines of transplant patients

Specimen	Time of appearance (weeks) of JCV antigen or CPE	
	UD cells	PHFG cells
PML brain		
no. 1	4	3
no. 2	8[a]	3
no. 3	8[a]	3
no. 4	Neg.[b]	Neg.
no. 5	9[a]	3
Normal brain	Neg.	Neg.
Urine		
no. 1	3	Not tested
no. 2	5	Not tested
no. 3	8[a]	Not tested

[a] Cultures which were positive for virus after an additional one to three passages.

[b] Neg., None appearing.

urine available were not sufficient for inoculation of PHFG cells.

It is not surprising that UD cells support papovavirus multiplication, as virus has frequently been identified in transitional urothelial cells in urine sediment (1). Although the precise in vivo origin of the viable cells cultured from urine is unclear, these cells are probably exfoliated from the renal tubules and pelvis, ureter, bladder, and urethra (3). It is possible that the UD cell culture system represents the in vitro correlate of the in vivo target cells of JCV and BK virus and may provide an alternative system for isolation and characterization of the human papovaviruses.

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