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Cloned Human Polyomavirus JC DNA Can Transform Human Amnion Cells

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The genome of the human polyomavirus JC (Mad-1 strain) was molecularly cloned in *Escherichia coli* by using the plasmid vector pBR322. Recombinant DNA molecules were constructed with the entire JC genome inserted either at its unique *Eco*RI site at 0.0 map units or at its unique *Bam*HI site at 0.51 map units. Viral DNA from each of these recombinant plasmids was capable of transforming human amnion cells, and cell lines established from transformed foci were positive for JC tumor antigen as assayed by indirect immunofluorescence.

The human polyoma virus JC was first isolated in 1971 from the brain of a patient with progressive multifocal leukoencephalopathy (22). Serological evidence indicates that infection with JC virus is widespread and that seroconversion usually occurs in childhood with no recognized symptoms (2, 20). The virus has subsequently been isolated from or identified in at least 40 brain specimens of patients with progressive multifocal leukoencephalopathy, strongly implicating JC virus as the etiological agent in this rare demyelinating disease (12, 21, 23). JC virus has also been identified in the urine of renal transplant recipients on immunosuppressive therapy (8, 11) and in that of normal pregnant women during uncomplicated gestations (4). This human virus has generated interest not only because of its association with progressive multifocal leukoencephalopathy but also because of its demonstrated oncogenicity. JC virus induces a variety of tumors in hamsters (24, 30, 32) and brain tumors in owl monkeys (16). Recently, it has been shown to transform primary hamster brain cells in tissue culture (7).

The highly restricted host range of JC virus in tissue culture has made the propagation of this virus exceedingly difficult. JC virus replicates well only in primary cultures of spongioblast-rich human fetal glial cells (PHFG) (22). In addition, JC virus can replicate less efficiently in primary human amnion cells (28), human endothelial cells (5), and human embryonic kidney cells (19). Consequently, opportunities to study the biology and pathogenicity of this virus have been restricted by the limited amount of JC virus and JC DNA available.

To partially circumvent this limitation, we have molecularly cloned the entire genome of the Mad-1 strain of JC virus in *Escherichia coli* K-12 by using the certified plasmid vector pBR322. JC (Mad-1) DNA purified from virions was generously supplied by Jonathan Martin. This preparation of DNA had been purified from virus propagated at low multiplicity in PHFG cell cultures and is the same preparation of particularly homogeneous DNA which had been previously described in experiments from our laboratory aligning the JC genome with simian virus 40 and the human polyomavirus BK DNAs (15). In separate experiments, JC (Mad-1) DNA was cloned at either the single *Eco*RI site located at 0.0 map units (17) or at the single *Bam*HI site located at 0.51 map units (Fig. 1). Appropriately cleaved linear JC (Mad-1) DNA molecules were ligated to pBR322 at either the *Eco*RI site or the *Bam*HI site as previously described (14). The products of these ligation reactions were then used to transform susceptible *E. coli* K-12 (strain HB101) bacteria (33), and ampicillin-resistant transformants were selected for further characterization. Approximately 100 bacterial colonies transformed by the *Eco*RI ligation mixture were screened for JC DNA sequences by a modification of the colony filter hybridization technique (10). DNA from eight colonies hybridized to JC viral DNA labeled in vitro by nick translation (26). Bacteria containing a recombinant plasmid with an insert at the *Bam*HI site in pBR322 are characterized by ampicillin resistance and tetracycline sensitivity, since an insert at this site interrupts the pBR322 tetracycline resistance gene (1). Of 72 bacterial colonies transformed to ampicillin resistance by the ligation products of *Bam*HI-cleaved pBR322 and JC (Mad-1) DNAs, 12 were found to be tetracycline sensitive.

Plasmid DNA was prepared from chloramphenicol-amplified bacterial cultures (3) and analyzed by restriction endonuclease cleavage and agarose gel electrophoresis (Fig. 2). *Eco*RI cleav-

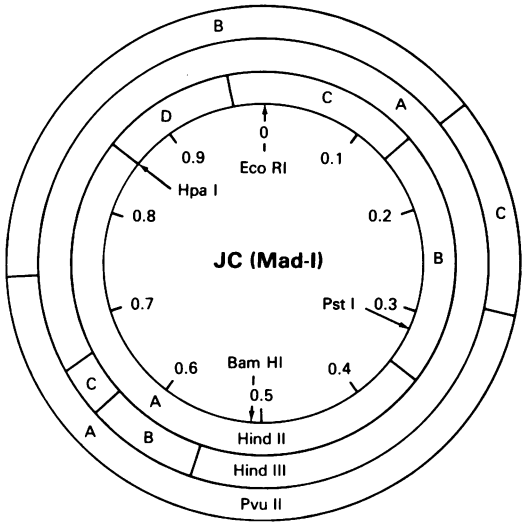

FIG. 1. *Restriction endonuclease map of JC (Mad-1) DNA. Map units are expressed as a fraction of the circular JC genome commencing at the single EcoRI cleavage site (17). The PstI, HpaI, BamHI, HindIII, and HindII sites were determined by Martin et al. (17). The PvuII sites were determined from the recombinant DNAs described in this paper (Engel and Howley, unpublished data).*

age of plasmid DNA from each of the eight bacterial clones thought to contain JC DNA inserted at the *Eco*RI site produced only two restriction fragments. One DNA fragment comigrated with full-length linear JC (Mad-1) DNA (5.0 kilobases [kb]), and the other comigrated with linear pBR322 DNA (4.3 kb) (Fig. 2, lane f). The orientation of the JC (Mad-1) DNA insert was determined by digestion of the hybrid plasmid DNAs with *Pst*I which cleaves pBR322 once and JC (Mad-1) DNA once at 0.32 map units (17). Cleavage of a hybrid plasmid containing a JC (Mad-1) DNA insert in one of the two possible orientations resulted in joint fragments containing both pBR322 and JC DNA of 6.9 and 2.4 kb in size, whereas cleavage of the hybrid plasmid in the alternate orientation resulted in joint fragments of 5.1 and 4.2 kb. An analysis of the orientation of the recombinant DNA molecules [pJC(2-4) and pJC(2-5)] is shown in Fig. 2. Although cleavage of pJC(2-5) (Fig. 2, lane k) with *Pst*I resulted in fragments of approximately 6.9 and 2.4 kb, *Pst*I cleavage products of pJC(2-4) DNA (Fig. 2, lane h) included fragments of approximately 5.1 and 4.2 kb, indicating that these two recombinant plasmids contain the JC viral DNA insert in opposite orientations. An additional band comigrating with full-length linear JC (MAD-1) DNA could be detected after cleavage of pJC(2-4) with *Bam*HI (lane g) and *Pst*I (lane h). The presence of this fragment after cleavage with a restriction enzyme which recognizes a single site in the JC genome indicates an oligomeric head-to-tail insert of JC (Mad-1) DNA in this clone. Size analysis of uncleaved pJC(2-4) DNA on agarose gels indicated that this plasmid contains two viral genomes per recombinant plasmid (data not shown). Hybridization of a ^{32}P-labeled JC DNA probe to the appropriate DNA fragments after transfer of DNA from the gel shown in Fig. 2A to a nitrocellulose filter confirmed the presence of JC (Mad-1) DNA sequences (Fig. 2B).

Plasmid DNA was similarly prepared from chloramphenicol-amplified bacterial cultures of four ampicillin-resistant, tetracycline-sensitive isolates obtained after transformation of *E. coli* HB101 with the *Bam*HI pBR322–JC (Mad-1) DNA ligation mixture. Three of four isolates did not contain a viral DNA insert, whereas one [pJC(1–4)] was found to contain a full-length JC (Mad-1) DNA insert after digestion of the recombinant plasmid DNAs with *Bam*HI. Cleavage of this DNA with *Pst*I resulted in two fragments of 5.1 and 4.2 kb (Fig. 2, lane e), indicating the orientation of the JC DNA insert. The presence of JC sequences in the insert was also confirmed by specific hybridization of a ^{32}P-labeled JC DNA probe to the appropriate DNA fragments denatured in situ and transferred to a nitrocellulose filter (Fig. 2B).

The biological activity of cloned JC (Mad-1) DNA was assayed in primary human amnion cells (HEM Research, Inc., Rockville, Md.). Recombinant plasmid DNA cleaved with either *Eco*RI or *Bam*HI to yield full-length linear viral and pBR322 DNAs was used to transfect primary human amnion cell cultures by both the DEAE-dextran method (18) and the calcium precipitation technique (9) since Frisque and his co-workers recently showed that infection of PHFG cell cultures with JC viral DNA was more efficient with the calcium technique (6). Human amnion cell cultures (60-mm dishes) infected by either technique with 1 μg of JC-pBR322 recombinant plasmid DNAs cleaved with *Bam*HI or *Eco*RI or with 1 μg of plasmid DNA alone did not display any specific cytopathic effect. In addition, no hemagglutinating activity could be detected in the culture media even 4 weeks after infection, indicating the absence of significant JC virus production. Infected cells were separately examined for JC T- and V-antigens 2 weeks after infection and found by indirect immunofluorescence to be negative.

Approximately 3 to 4 weeks after infection, transformed foci were noted in the primary human amnion cultures transfected by the calcium precipitation technique with *Bam*HI-cleaved pJC(1-4) DNA and *Eco*RI-cleaved pJC(2-5)

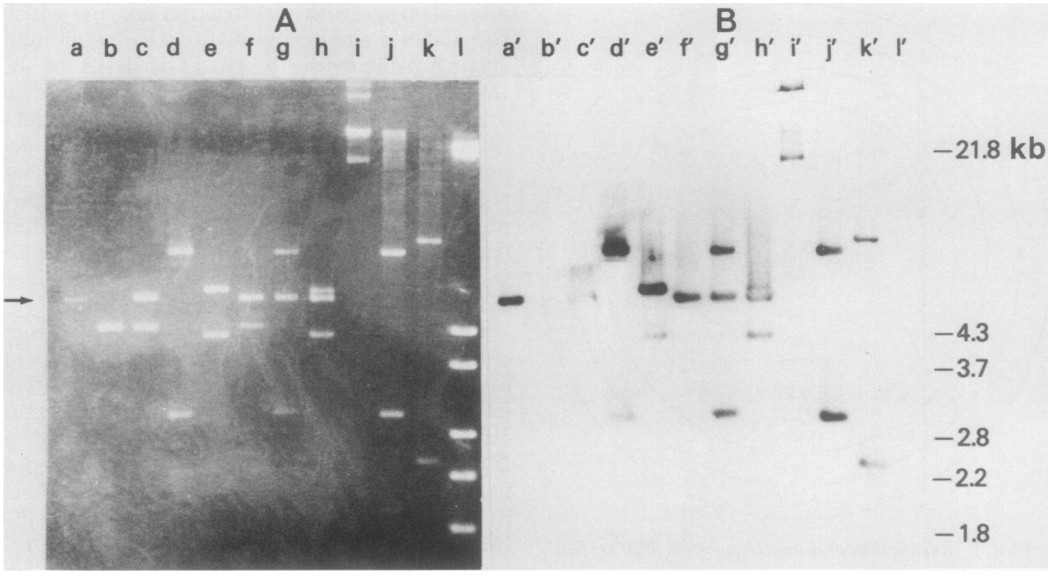

FIG. 2. *Analysis of recombinant JC (Mad-1)-pBR322 DNA molecules. DNA fragments (0.5 μg per lane) generated by the indicated restriction endonuclease cleavage of the viral or plasmid DNA molecules were separated in a 1% agarose (Seakem) gel and, after staining with ethidium bromide (0.5 μg/ml) for 20 min, were visualized with shortwave UV light (A). After photography, the DNA was denatured in situ, blotted onto nitrocellulose paper (27), and hybridized to a nick-translated (26) ^{32}P-labeled JC (Mad-1) DNA probe (specific activity, 5 × 10^7 cpm/μg) as previously described (13) (B). The autoradiograph (B) is shown after 3 h of exposure at room temperature. Lane a contains form III (BamHI-linearized) JC (Mad-1) DNA as indicated by the arrow. Lane b contains BamHI-linearized pBR322 DNA. Lanes c, d, and e contain pJC(1-4) DNA cleaved with BamHI, EcoRI, and PstI, respectively. Lanes f, g, and h contain pJC(2-4) DNA cleaved with EcoRI, BamHI, and PstI, respectively. Lanes i, j, and k contain pJC(2-5) DNA cleaved with EcoRI, BamHI and PstI, respectively. In this gel, cleavage of pJC(2-5) with EcoRI did not occur; however, in other experiments, limit digestion of this DNA with EcoRI resulted in two fragments of 5.0 and 4.3 kb. Lane l contains the EcoRI-generated fragments of adenovirus type 2 which serve as size markers.*

DNA. For each DNA, the efficiency of transformation in these experiments was approximately 5 foci per μg of viral DNA. No foci were noted in cells transfected with vector DNA or the carrier salmon sperm DNA alone. Cloned cell lines were established from these foci and shown by indirect immunofluorescence to contain JC T-antigen (Fig. 3), although they were negative for JC V-antigen. Recent experiments have indicated the presence of full-length JC DNA sequences in a circular episomal state in each of these cell lines (data not shown). A more complete biological and biochemical analysis of these transformed cell lines is in progress.

Our inability to productively infect primary human amnion cells with the cloned JC DNAs was unexpected since we had previously shown that at least one stock of JC (Mad-1) could replicate efficiently in these cells (28). It seems unlikely that the cloned JC genomes described here would each be unable to productively infect permissive cells, since the restriction endonuclease patterns of each of the independently cloned JC DNAs are identical to each other and to that of JC (Mad-1) viral DNA (17). Recent experiments in our laboratory suggest that primary human amnion cells do not support the productive replication of all stocks of JC virus equally. In fact, the original DNA from which the hybrid clones were made was not infectious when transfected on human amnion cells. Therefore, the infectivity of our cloned JC DNAs should ideally be tested in PHFG cells since JC DNA is infectious in these cells (6) and since the JC virus preparation from which our cloned DNAs had been made was propagated in PHFG cells. Because of the lack of availability of these cells, we have thus far not been able to assay the infectivity of cloned JC (Mad-1) DNAs on PHFG cells.

Although transformation of human fetal astrocytes (31) and human endothelial cells (5) by JC virus has previously been reported, permanent transformed lines could not be established. In addition, we have recently become aware that F. O'Neill has also transformed human amnion cells with JC virus (F. O'Neill, personal communication). Transformation of permissive primary cells has also been described for the human

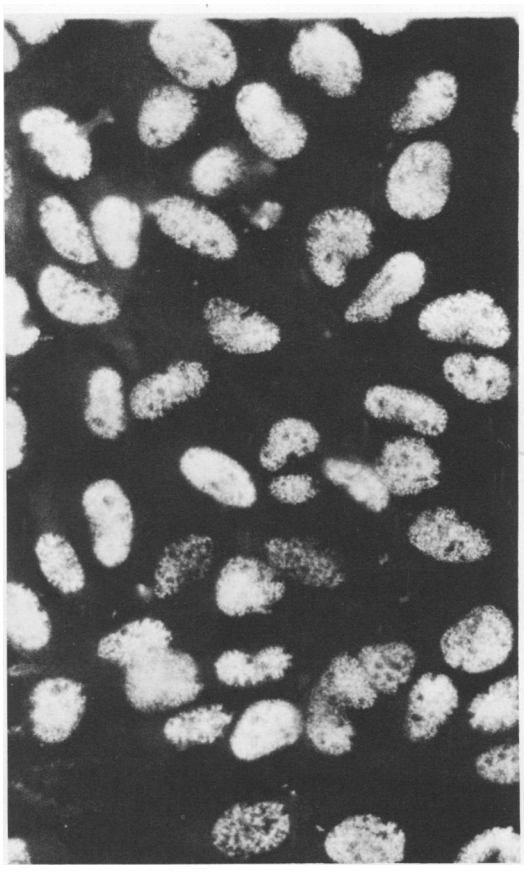

FIG. 3. *Immunofluorescent staining for JC T-antigen in a cloned human amnion cell line transformed by pJC(1-4) DNA cleaved with BamHI.*

polyomavirus BK in human embryonic kidney cells (25) and in human fetal brain cells (29). In each of these cases with human polyomavirus BK, cells surviving the initial lytic infection formed transformed foci and, like the JC virus-transformed primary human amnion cells described here, contained nonintegrated episomal viral DNA. The biological and biochemical characteristics of these JC virus-transformed primary human amnion cells are currently under investigation.

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