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Direct Isolation and Characterization of JC Virus from Urine Samples of Renal and Bone Marrow Transplant Patients

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JC virus DNA was extracted from urine-derived cells of bone marrow and renal transplant patients and cloned directly into the plasmid vector pBR322. These clones represent the first JC virus isolates obtained directly from individuals that did not have progressive multifocal leukoencephalopathy (PML). Three of the clones appeared to be identical to the prototype JC virus Mad 1, and the fourth clone was identical to the type II JC virus variant Mad8-Br. Importantly, the same JC virus strains have been identified both in the urine of non-PML patients and in the brain tissue of PML patients. These results indicate that different organs may be infected with the same JC virus subtype and implies that an adaptation process involving the alteration of viral regulatory signals is not required in the pathogenesis of PML. Furthermore, both a type I and a type II variant were obtained from the same patient, suggesting that an individual may be infected with more than one strain of JC virus at a given time.

JC virus (JCV) is the causative agent of progressive multifocal leukoencephalopathy (PML), a demyelinating disease of the central nervous system (34). Although the incidence of PML is low, the number of reported PML cases has increased in recent years, due in part to the occurrence of the acquired immunodeficiency syndrome (AIDS) epidemic. A significant number of the AIDS patients that exhibit neurological complications (3 to 5%) have been diagnosed as having PML (20). Very little is known about the transmission and pathogenesis of a JCV infection. Serological studies indicate that JCV is widespread in the population and that primary infection usually occurs at an early age (33). Antibody levels are maintained throughout life, which suggests that JCV may establish a persistent infection. JCV DNA has been detected in the kidney of individuals without PML and in the kidney, lung, liver, spleen, and lymph nodes of PML patients (4, 8, 13). The virus has also been found in the urine of healthy women during pregnancy (6) and in the urine of organ transplant patients during immunosuppressive therapy (3, 11, 16, 17), suggesting that the virus persists in the renal tissue and can be reactivated under certain conditions. In a rare number of immunocompromised patients, the reactivated virus may reach the central nervous system and infect brain tissue to cause the demyelinating disease. A recent report suggests that JCV may productively infect mononuclear cells in the bone marrow (18). Once differentiated, the infected B cells may transport the virus across the blood-brain barrier, allowing the virus to infect the oligodendrocytes.

JCV has an extremely restricted host range in vitro; the prototype Mad 1 strain replicates well only in primary human fetal glial (PHFG) cells (32) and PHFG cells transformed by origin-defective simian virus 40 (SV40) (SVG cells [22]) or JCV (POJ cells [23]). The regulatory sequences of isolates of JCV obtained from the brain tissue of different PML patients show considerable variation (12, 26). While the Mad 1 promoter and enhancer signals function well only in glial cells (19), the transcriptional elements of some JCV variants show a wider range of activity (F. White III, M. Cannella, and R. J. Frisque, manuscript in preparation). It has been hypothesized that genetic alterations in the regulatory region may be part of the pathogenic process by which JCV changes from a kidney- to a brain-adapted virus (7, 10, 21). If adaptation does occur, the regulatory signals of strains of JCV isolated from the urine of individuals without PML might differ significantly from those of JCV isolated from the brain tissue of PML victims. To pursue this possibility, we isolated JCV from "normal" individuals. This report describes the first molecular cloning of JCV DNA extracted directly from urine-derived cells of renal and bone marrow transplant patients without PML.

A total of 917 urine samples from 310 patients were collected for evaluation of polyomavirus. The 660 specimens from 175 renal transplant recipients constituted the majority of the samples. The remaining urine samples were collected from 53 immunodeficient children, 47 patients with connective tissue disorders who were receiving immunosuppressive therapy, 23 bone marrow transplant recipients, and 12 patients with urological ailments. Although the specific type of urinary cells infected with JCV was not identified, urine-derived transitional epithelial cells with nuclear inclusions containing polyomaviruses are frequently observed in patients with viruria (6). Thirteen specimens were found to be positive for JCV by methods described below and were available in sufficient quantity for cloning experiments. All specimens except single samples from a marrow transplant recipient and a connective tissue disease patient were from renal transplant recipients. JCV DNA was successfully cloned from 2 of these 13 specimens. Descriptions of the case histories of the two patients follow.

Patient 86-121 was a 41-year-old male with acute myelogenous leukemia who underwent allogeneic bone marrow transplantation. Cyclosporin A was administered during the posttransplant period. On day 27 posttransplant, he developed graft-versus-host disease, and steroid therapy was added to the treatment regimen. A single urine specimen collected from this patient on day 41 after transplantation was positive for JCV.

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TABLE 1. History of JCV(N) DNAs

Patient	Transplant procedure	Cloned DNA	Variant type[a]
86–121	Bone marrow	JCV(N1)	I
		JCV(N2)	I
86–298	Renal	JCV(N4)	I
		JCV(N5)	II

[a] Type I DNAs have a duplicated TATA box, lack an Sp1-binding site consensus sequence, and contain a 98-bp tandem repeat. Most type II variants differ from one another, but each has lost a copy of the TATA sequence, acquired one to two copies of the Sp1 consensus sequence, and diverged from the prototype enhancer element sequence at the same nucleotide (26).

Patient 86-298 was a 51-year-old male with a long history of insulin-dependent diabetes mellitus and renal failure who had received a renal allograft 15 years previously. Since the time of transplantation, prednisone and azathioprine had been administered daily to prevent graft rejection. The JCV-positive specimen was collected during a routinely scheduled clinic visit. A specimen collected 6 months later was negative for JCV and BK virus (BKV). Urine specimens that were collected and examined during the period from 4 to 7 years following transplantation had cytologic evidence of polyomavirus infection.

Viral DNA in urinary sediments in samples from immunosuppressed patients was initially detected by a filter in situ hybridization technique (1) with a mixture of ^{32}P-labeled JCV and BKV probes. This method permitted the efficient examination of many samples. JCV was identified in positive samples by Southern hybridization (37). Viral DNA was extracted from cells present in the urine samples of immunosuppressed patients and was digested with *Bam*HI and *Hha*I. The fragments were separated by agarose gel electrophoresis, transferred to nylon membranes, and hybridized to ^{32}P-labeled JCV and BKV probes (2). JCV and BKV DNAs could be differentiated by their restriction digest patterns and the intensity of the hybridization signal with each probe. Both viral DNAs contain a *Bam*HI cleavage site, and digestion with this enzyme linearized the genomes. BKV has a single *Hha*I cleavage site, and digestion with this enzyme resulted in fragments of 1.3 and 3.9 kilobases (kb), whereas JCV does not have an *Hha*I cleavage site and appeared as a single 5.1-kb band. Viral DNAs obtained from JCV-positive specimens (13 samples) were used for cloning directly into the plasmid vector pBR322 at the *Eco*RI site, and four recombinant clones were obtained from two specimens. These recombinants are referred to as pJCV(N), which indicates JCV DNA isolated from non-PML patients and cloned into pBR322; JCV(N) refers to the viral DNAs removed from the plasmid. Two of the clones, pJCV(N1) and pJCV(N2), were obtained from a bone marrow transplant patient, and clones pJCV(N4) and pJCV(N5) were obtained from a renal transplant patient (Table 1). A fifth clone was obtained that appeared to contain BKV sequences as determined by restriction digests (data not shown).

Full-length JCV DNA could be recovered from each pJCV(N) clone by *Eco*RI digestion. The enzymes *Nco*I, *Bgl*II, *Pvu*II, *Sac*I, and *Hae*III were used to characterize the recombinant DNAs (Fig. 1). Restriction enzyme patterns were compared with those obtained for the prototype JCV clone pMad1-TC (24) and a variant of JCV, pMad8-Br (25). These two viral DNAs were obtained directly from the brain tissue of different PML patients. The *Nco*I digest provided preliminary information on the size of the regulatory region. There were two *Nco*I sites in the pMad1-TC and pMad8-Br

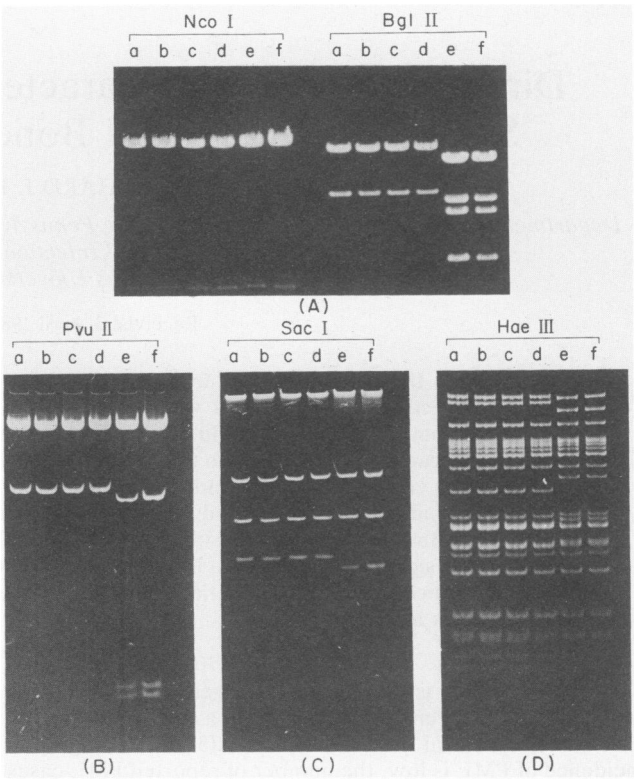

FIG. 1. Comparison of restriction enzyme digestion patterns for JCV(N) DNAs: pMad1-TC (a), pJCV(N1) (b), pJCV(N2) (c), pJCV(N4) (d), pJCV(N5) (e), and pMad8-Br (f). *Nco*I and *Bgl*II digestion products (A) were separated on a 0.8% agarose gel. Restriction fragments obtained by digestion with *Pvu*II (B), *Sac*I (C), and *Hae*III (D) were separated on either a 9% (*Sac*I) or 7% (*Pvu*II, *Hae*III) polyacrylamide gel. The cleavage pattern obtained with each of these restriction enzymes is discussed in the text.

DNAs, as well as in each of the pJCV(N) DNAs. The length of the smaller *Nco*I fragment was 425 base pairs (bp) for pMad1-TC and 434 bp for pMad8-Br; this size difference was not distinguishable on the agarose gel. The pJCV(N) DNAs produced *Nco*I fragments that were similar in size. *Bgl*II cleaved pMad1-TC into two fragments; pJCV(N1), pJCV(N2), and pJCV(N4) DNAs showed the same *Bgl*II restriction pattern. pJCV(N5) was found to have two additional *Bgl*II sites, which is characteristic of pMad8-Br DNA. The DNA from different strains of JCV display one of two *Pvu*II restriction patterns (12). Single-base substitutions at nucleotides 85 and 160 (A to G and C to G, respectively) created two new *Pvu*II sites in both pJCV(N5) and pMad8-Br, generating the additional *Pvu*II fragments seen with many type II variants of JCV (12). Additionally, the 664-bp *Pvu*II fragment of pMad8-Br and pJCV(N5) migrated differently from the corresponding fragment of pMad1-TC and pJCV(N1), pJCV(N2), and pJCV(N4) on an acrylamide gel. A previous report suggested that this was due to a deletion of approximately 75 bp in the pMad8-Br genome (12). However, the 664-bp *Pvu*II fragment from each of these DNAs comigrated on an agarose gel, indicating that the fragments were equivalent in size. The 664-bp *Pvu*II fragment of pMad1-TC, which encodes carboxy-terminal sequences of T antigen, contains a region of bent DNA, and nucleotide substitutions in this region of the Mad8-Br early coding sequence reduce the degree of DNA bending (D. King, M.S.

thesis, the Pennsylvania State University, University Park, Pa., 1987). These alterations account for the different polyacrylamide gel migration patterns seen for pMad8-Br and probably for pJCV(N5) DNA relative to that for pMad1-TC DNA. *Sac*I cut one time in each of the tandem repeats, illustrating the distinctive length of the enhancer element (98 bp in Mad 1 and 83 bp in Mad8-Br [26]). The *Hae*III digest gave a readily identifiable pattern for the JCV genome. The *Hae*III restriction patterns for the pMad1-TC, pMad8-Br, and the four pJCV(N) DNAs were all similar; pJCV(N1), pJCV(N2), and pJCV(N4) were identical to pMad1-TC, and pJCV(N5) was the same as pMad8-Br.

The regulatory region (0.67 to 0.73 map units) of the JCV genome has been shown to be hypervariable (12, 25, 26, 35). Because the noncoding region influences both the tissue and host specificity of the virus (9, 19), the nucleotide sequence of this region was determined for the four JCV(N) isolates. A restriction fragment produced by a *Hin*dIII-*Nco*I digest (nucleotides 5112 to 275 for Mad 1 [10]) and encompassing the replication origin and the promoter and enhancer elements was sequenced by the method of Maxam and Gilbert (28). This segment of the regulatory region was identical for JCV(N1), JCV(N2), and JCV(N4) and Mad 1 (10). JCV(N5) regulatory sequences shared 100% homology with those of Mad8-Br (26).

The lytic and DNA-replicating activities of JCV(N) DNAs were tested in PHFG and human embryonic kidney (HEK) cells. These cells were prepared from the tissue of aborted fetuses and maintained as described earlier (34). The viral DNAs used in these experiments were separated from plasmid sequences and recircularized by ligation. Each DNA was checked by agarose gel electrophoresis to ensure that equal quantities of ligated DNA were used to transfect the cells.

Lytic experiments were first conducted in PHFG cells, the most efficient culture system for the propagation of JCV. Cells growing on 12-mm cover slips were transfected with 0.05 μg of each viral DNA. The induction of T antigen was used to monitor the course of viral activity; T antigen was detected by indirect immunofluorescence with anti-SV40 or anti-JCV T antigen antiserum (5). JCV(N1), JCV(N2), JCV(N4), and Mad 1 expressed low levels of T antigen as early as day 3, and by day 21 large patches of T-antigen-containing cells were observed in the cultures, indicating that secondary infection had occurred. T-antigen expression in JCV(N5)- and Mad8-Br-transfected cells was observed only on day 3 posttransfection; cells were not producing detectable levels of viral proteins on day 14 or 21 posttransfection.

To determine whether infectious virions were produced in the transfected cells, lysates of these cells were prepared and added to fresh PHFG cells. The appearance of T antigen in these cells was taken as evidence that infectious virions were present in the lysate. These experiments indicated that transfection of PHFG cells with JCV(N1), JCV(N2), JCV(N4), and Mad 1 generated viable virus (Table 2). However, lysates from JCV(N5)- and Mad8-Br-transfected cells failed to induce T antigen when passaged onto fresh PHFG cells, suggesting that these viruses do not pass through a complete lytic cycle or that the amount of virus produced is too low to be detected by the immunofluorescence assay.

To test the replicative activity of the JCV(N) DNAs, PHFG cells were transfected with Mad 1, Mad8-Br, and the four JCV(N) DNAs, and low-molecular-weight DNA was recovered from these cells at several time points (15). Input DNA and newly replicated DNA were distinguished by using the *Dpn*I replication assay. Newly synthesized DNA could be detected as early as day 7 for JCV(N1)-, JCV(N2)-, JCV(N4)-, and Mad 1-transfected cells (Fig. 2). For these isolates, the amount of replicated DNA increased steadily until the final time point (day 21 posttransfection). The replication activities of JCV(N1), JCV(N2), and JCV(N4) were approximately equivalent to that of Mad 1. Both JCV(N5) and Mad8-Br DNAs replicated less efficiently than the other DNAs. The amount of replicated DNA peaked on day 14 and then declined.

TABLE 2. Lytic and DNA replication activities of JCV(N) DNAs in PHFG and HEK cells[a]

DNA	PHFG cells				HEK cells			
	Lytic activity	Replication			Lytic activity	Replication		
		Day 7	Day 14	Day 21		Day 7	Day 14	Day 21
Mad 1	+	1.00	1.00	1.00	−	1.00	1.00	1.00
JCV(N1)	+	1.01	0.91	1.30	−	0.90	0.84	1.40
JCV(N2)	+	1.09	0.82	1.10	−	0.92	0.74	1.11
JCV(N4)	+	1.00	0.92	1.08	−	1.00	1.15	1.11
JCV(N5)[b]	−	0.02	0.02	0.03	−	0.01	<0.01	0.04
Mad8-Br[b]	−	0.01	0.03	0.06	−	0.01	<0.01	0.01

[a] For lytic activity, PHFG and HEK cells were transfected with JCV DNAs by the DEAE-dextran procedure (36). On day 28 posttransfection, cells growing on 35-mm plates were frozen, thawed, and gently shaken to lyse cells and free potential virus. The extracts were sonicated and added to fresh PHFG or HEK cells growing on 12-mm cover slips. The induction of viral T antigen in these cells was detected by immunofluorescence and indicated the presence of infectious virions in the lysate. The data presented here were obtained on days 14 and 21 postinfection. For replication activity, bands representing *Dpn*I-resistant, full-length JCV DNA were excised from the nitrocellulose filter and counted in an LS 5801 liquid scintillation counter (Beckman Instruments) and/or autoradiograms were scanned densitometrically with an Ultroscan XL enhanced laser densitometer (LKB Instruments, Inc.). All values are expressed relative to the replication activity of Mad 1 (arbitrarily given a value of 1.00). To minimize potential differences in transfer and hybridization efficiencies that might affect the comparisons, all DNA samples representing a single time point (7, 14, or 21 days posttransfection) were run on the same gel to make these measurements. Values obtained at the day 21 time point for the glial cell replication experiment were approximately 6,000-fold greater than those obtained in the HEK replication experiment.

[b] In independent experiemnts, MadB-Br and JCV(N5) replicated from very low levels to undetectable levels in HEK cells (this table and data not shown).

Lytic and DNA replication experiments similar to those described above were carried out in HEK cells. All JCV DNAs consistently gave negative results for the lytic assay in these cells; lysates prepared from transfected HEK cells failed to induce T antigen when added to fresh HEK cultures (Table 2). Furthermore, Southern blot analysis (37) of low-molecular-weight DNA extracted from these secondarily infected cells failed to detect the presence of JCV DNA, suggesting that infectious virus was not produced in HEK cells (data not shown). However, results from the *Dpn*I assay indicated that Mad 1 and JCV(N1), JCV(N2), and JCV(N4) replicated to low levels in HEK cells. Mad8-Br and JCV(N5) did not appear to replicate to significant levels in HEK cells.

Organ transplant patients often excrete JCV or BKV or both in their urine during immunosuppression (3, 11, 16, 17). We isolated four clones of JCV DNA from urine-derived cells; two of these clones, JCV(N1) and JCV(N2), were obtained from a bone marrow transplant patient, and two other clones, JCV(N4) and JCV(N5), were isolated from a renal transplant patient. These DNAs represent the first JCV isolates cloned directly from non-PML patients.

It has been suggested that JCV found in the kidneys of

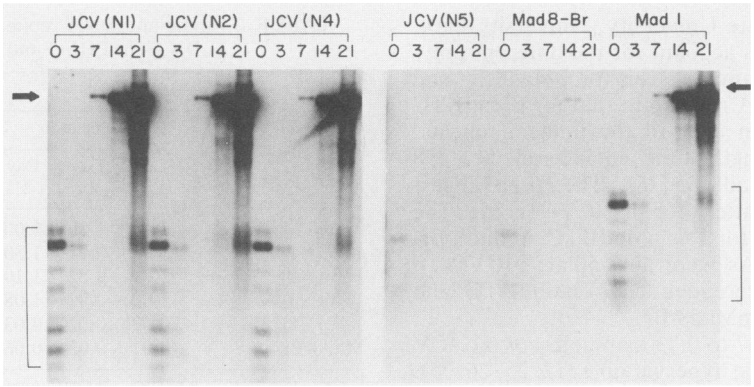

FIG. 2. Replicative activity of JCV(N) DNAs in PHFG cells. Viral DNA was extracted from cells 0, 3, 7, 14, and 21 days posttransfection (15), as indicated above the lanes. Samples were digested with *Eco*RI and *Dpn*I, separated on a 0.8% agarose gel, and analyzed by the technique of Southern (37). The DNAs were hybridized to a radioactively labeled pMad1-TC probe (Pharmacia Oligolabeling Kit) and visualized by autoradiography. The arrow and brackets indicate the positions of the *Eco*RI- (linear) and *Eco*RI-*Dpn*I-cleaved JCV DNA, respectively. Lanes 0 contain input DNA extracted from the cells immediately after transfection; in this experiment, reduced levels of input DNA were apparent for the Mad8-Br and JCV(N5) samples. However, in each of the four replication experiments in PHFG cells, the largest of the *Dpn*I bands in the Mad 1 and Mad8-Br lanes differed in intensity due to less efficient transfer and hybridization of the smaller Mad8-Br fragment. In each case, replication of the Mad8-Br and JCV(N5) DNAs was less efficient than that of the other DNAs, reached a peak at 14 days, and declined by 21 days posttransfection.

normal individuals might differ significantly from JCV found in the brain tissue of PML victims (10, 21, 29). Isolates of JCV obtained from brain tissue of different PML patients are highly variable in the sequence of their regulatory elements (12, 26, 35). From the influence that these sequences have on the tissue and host specificity of the polyomaviruses (14, 31, 38), it has been suggested that genetic alterations in the regulatory region may be partially responsible for the adaptation of JCV to growth in cells of neural origin (7, 10, 21). The DNAs of our four isolates were characterized by restriction enzyme digestion of the entire genome and by sequence analysis of the enhancer and promoter region. We concluded that three of these clones, JCV(N1), JCV(N2), and JCV(N4), were genetically equivalent to the prototype strain of JCV, Mad 1. The fourth clone, JCV(N5), was indistinguishable from the JCV variant Mad8-Br. These results indicate that JCV found in the urine of some non-PML individuals is identical to JCV isolated from the brain tissue of PML patients. Furthermore, since different organs may be infected with the same strain of JCV, it appears that an adaptation process, involving alterations of viral regulatory signals to allow growth in a new tissue, is not required in the pathogenesis of PML.

Propagation of JCV in tissue culture prior to cloning gives rise to a heterogeneous population of recombinant DNAs (27), while JCV cloned directly from the brain tissue of PML patients has generally been found to be homogeneous for a single individual (12). Dorries (7) reported that although JCV isolates from the brain and kidney of a single PML patient differed, recombinant DNAs were indistinguishable when cloned from the same organ. Our isolates JCV(N4) and JCV(N5) were cloned directly from the urine sample of a single renal transplant patient and did show variability in their genomes, in both the regulatory and coding regions, indicating that either coinfection with multiple JCV strains or variation in a single strain had occurred within this patient.

JCV has a very restricted host range in vitro, with prototype JCV replicating well only in normal and transformed PHFG cells. The virus has been shown to replicate in HEK cells, but only after undergoing extensive genomic rearrangements following several passages of the virus in these cells in culture (30). Our lytic and DNA replication assays have shown that JCV(N1), JCV(N2), and JCV(N4), as well as Mad 1, are viable in PHFG cells. JCV(N5) and Mad8-Br replicated their DNAs less efficiently in these cells and did not produce detectable levels of infectious virions. It is not clear whether these differences are due to changes in the regulatory or coding regions of the Mad8-Br and JCV(N5) genomes. Preliminary studies in POJ cells indicate that Mad8-Br replicates in these cells to a level equivalent to that of Mad 1 (unpublished results). This result may be due to an increase in the quantity of T antigen (provided in *trans* by the POJ cells) or may indicate a defect in the T antigen of Mad8-Br. These results also support the possibility that the persistence of Mad8-Br and JCV(N5) in vivo requires the complementing activity of a second strain of JCV.

For JCV(N1), JCV(N2), JCV(N4), and Mad 1, the level of replicative activity in HEK cells was considerably reduced compared with that observed in PHFG cells. Mad8-Br and JCV(N5) did not replicate to detectable levels in HEK cells, and production of infectious virus could not be demonstrated for any of these isolates in HEK cells. The kidney cell type(s) in which JCV persists in vivo is not known. It is possible that the cell type is not amenable to culturing under our conditions or is present only as a minor subclass in the kidney.

Our findings indicate that the same JCV which persistently infects kidney tissue has the potential to be lytically active in the brain, and therefore alterations to the viral genome are not required in the pathogenesis of PML.

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LITERATURE CITED

1. **Arthur, R. R., A. M. Beckmann, C. C. Li, R. Saral, and K. V. Shah.** 1985. Detection of the human papovavirus BK in urine of bone marrow transplant recipients: comparison of DNA hybridization with ELISA. J. Med. Virol. **16**:29–36.
2. **Arthur, R. R., and K. V. Shah.** 1988. Papovaviridae: the polyomaviruses, p. 317–332. *In* P. Halonen, E. Lennette, Jr., and F. Murphy (ed.), The laboratory diagnosis of infectious diseases: principles and practice, vol. 2. Springer-Verlag, New

York.
3. **Arthur, R. R., K. V. Shah, P. Charache, and R. Saral.** 1988. BK and JC virus infections in recipients of bone marrow transplants. J. Infect. Dis. **158**:563–569.
4. **Chesters, P. M., J. Heritage, and D. J. McCance.** 1983. Persistence of DNA sequences of BK virus and JC virus in normal human tissues and in diseased tissues. J. Infect. Dis. **147**: 676–684.
5. **Chuke, W.-F., D. L. Walker, L. B. Peitzman, and R. J. Frisque.** 1986. Construction and characterization of hybrid polyomavirus genomes. J. Virol. **60**:960–971.
6. **Coleman, D. V., M. R. Wolfendale, R. A. Daniel, N. K. Dhanjal, S. D. Gardner, P. E. Gibson, and A. M. Field.** 1980. A prospective study of human polyomavirus infection in pregnancy. J. Infect. Dis. **142**:1–8.
7. **Dorries, K.** 1984. Progressive multifocal leukoencephalopathy: analysis of JC virus DNA from brain and kidney tissue. Virus Res. **1**:25–38.
8. **Dorries, K., and V. ter Meulen.** 1983. Detection of papovavirus JC in kidney tissue. J. Med. Virol. **11**:307–311.
9. **Feigenbaum, L., K. Khalili, E. Major, and G. Khoury.** 1987. Regulation of the host range of human papovavirus JCV. Proc. Natl. Acad. Sci. USA **84**:3695–3698.
10. **Frisque, R. J., G. L. Bream, and M. T. Cannella.** 1984. Human polyomavirus JC virus genome. J. Virol. **51**:458–469.
11. **Gardner, S. D., E. F. D. Mackenzie, C. Smith, and A. A. Porter.** 1984. Prospective study of the human polyomaviruses BK and JC and cytomegalovirus in renal transplant recipients. J. Clin. Pathol. **37**:578–586.
12. **Grinnell, B. W., B. L. Padgett, and D. L. Walker.** 1983. Comparison of infectious JC virus DNAs cloned from human brain. J. Virol. **45**:299–308.
13. **Grinnell, B. W., B. L. Padgett, and D. L. Walker.** 1983. Distribution of nonintegrated DNA from JC papovavirus in organs of patients with progressive multifocal leukoencephalopathy. J. Infect. Dis. **147**:669–675.
14. **Herbomel, P., B. Bourachot, and M. Yaniv.** 1984. Two distinct enhancers with different cell specificities coexist in the regulatory region of polyoma. Cell **39**:653–662.
15. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365–369.
16. **Hogan, T. F., E. C. Borden, J. A. McBain, B. L. Padgett, and D. L. Walker.** 1980. Human polyomavirus infections with JC virus and BK virus in renal transplant patients. Ann. Intern. Med. **11**:178-183.
17. **Hogan, T. F., B. L. Padgett, D. L. Walker, E. C. Borden, and J. A. McBain.** 1980. Rapid detection and identification of JC virus and BK virus in human urine by using immunofluorescence microscopy. J. Clin. Microbiol. **11**:178–183.
18. **Houff, S. A., E. O. Major, D. A. Katz, C. V. Kufta, J. L. Sever, S. Pittaluga, J. R. Roberts, J. Gitt, N. Saini, and W. Lux.** 1988. Involvement of JC virus-infected mononuclear cells from the bone marrow and spleen in the pathogenesis of progressive multifocal leukoencephalopathy. N. Engl. J. Med. **318**:301–305.
19. **Kenney, S., V. Natarajan, D. Strike, G. Khoury, and N. Salzman.** 1984. JC virus enhancer-promoter active in human brain cells. Science **226**:1337–1339.
20. **Krupp, L. B., R. B. Lipton, M. L. Swerdlow, N. E. Leeds, and J. Llena.** 1985. Progressive multifocal leukoencephalopathy: clinical and radiographic features. Ann. Neurol. **17**:344–349.
21. **Loeber, G., and K. Dorries.** 1988. DNA rearrangement in organ-specific variants of polyomavirus JC strain GS. J. Virol. **62**:1730–1735.
22. **Major, E. O., A. E. Miller, P. Mourrain, R. G. Traub, E. deWidt, and J. Sever.** 1985. Establishment of a line of human fetal glial cells that supports JC virus multiplication. Proc. Natl. Acad. Sci. USA **82**:1257–1261.
23. **Mandl, C., D. L. Walker, and R. J. Frisque.** 1987. Derivation and characterization of POJ cells, transformed human fetal glial cells that retain their permissivity for JC virus. J. Virol. **61**: 755–763.
24. **Martin, J. D., K. H. Brackmann, B. W. Grinnell, R. J. Frisque, D. L. Walker, and M. Green.** 1982. Recombinant JC viral DNA: verification and physical map of prototype. Biochem. Biophys. Res. Commun. **109**:70–77.
25. **Martin, J. D., and G. C. Foster.** 1984. Multiple JC virus genomes from one patient. J. Gen. Virol. **65**:1405–1411.
26. **Martin, J. D., D. M. King, J. M. Slauch, and R. J. Frisque.** 1985. Differences in regulatory sequences in naturally occurring JC virus variants. J. Virol. **53**:306–311.
27. **Martin, J. D., B. L. Padgett, and D. L. Walker.** 1983. Characterization of tissue culture-induced heterogeneity in DNAs of independent isolates of JC virus. J. Gen. Virol. **64**:2271–2280.
28. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
29. **McCance, D. J.** 1983. Persistence of animal and human papovaviruses in renal and nervous tissues, p. 343–357. *In* J. L. Sever and D. L. Madden (ed.), Polyomaviruses and human neurological diseases. Alan R. Liss, Inc., New York.
30. **Miyamura, T., K. Yoshiike, and K. K. Takemoto.** 1980. Characterization of JC papovavirus adapted to growth in human embryonic kidney cells. J. Virol. **35**:498–504.
31. **Ondek, B., A. Shepard, and W. Herr.** 1987. Discrete elements within the SV40 enhancer region display different cell-specific enhancer activities. EMBO J. **6**:1017–1025.
32. **Padgett, B. L., C. M. Rogers, and D. L. Walker.** 1977. JC virus, a human polyomavirus associated with progressive multifocal leukoencephalopathy: additional biological characteristics and antigenic relationships. Infect. Immun. **15**:656–662.
33. **Padgett, B. L., and D. L. Walker.** 1973. Prevalence of antibodies in human sera against JC virus, an isolate from a case of progressive multifocal leukoencephalopathy. J. Infect. Dis. **127**: 467–470.
34. **Padgett, B. L., D. L. Walker, G. M. ZuRhein, A. E. Hodach, and S. M. Chow.** 1976. JC papovavirus in progressive multifocal leukoencephalopathy. J. Infect. Dis. **133**:686–690.
35. **Rentier-Delrue, F., A. Lubiniecki, and P. M. Howley.** 1981. Analysis of JC virus DNA purified directly from human progressive multifocal leukoencephalopathy brains. J. Virol. **38**:761–769.
36. **Sompayrac, L. M., and K. J. Danna.** 1981. Efficient infection of monkey cells with DNA of simian virus 40. Proc. Natl. Acad. Sci. USA **78**:7575–7578.
37. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
38. **Ter Schegget, J., C. J. A. Sol, E. Wouters Bann, J. van der Noordaa, and H. van Ormondt.** 1985. Naturally occurring BK virus variants (JL and Dik) with deletions in the putative early enhancer-promoter sequences. J. Virol. **53**:302–305.