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Sequence Rearrangement in JC Virus DNAs Molecularly Cloned from Immunosuppressed Renal Transplant Patients

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From nonimmunocompromised individuals, we have recently identified a possible archetypal JC virus DNA sequence from which various regulatory sequences of JC virus isolates derived from patients with progressive multifocal leukoencephalopathy (PML) could have evolved. In this study, we analyzed the regulatory sequences of JCV DNAs cloned from urine samples of a PML risk group (renal transplant patients on immunosuppressive therapy). A number of JC virus DNAs were molecularly cloned from virions excreted in the urine of eight patients. Furthermore, fragments containing the regulatory region were amplified by the polymerase chain reaction and subsequently molecularly cloned from cell-associated JC virus excreted in the urine of two patients. The regulatory regions in all clones were analyzed with restriction enzymes, and those in representative clones were sequenced. We found that clones with the archetypal regulatory sequence were predominant in all urine samples, but a few clones carried regulatory sequences that diverged from the archetypal sequence by deletion or duplication. The finding that sequence rearrangement in the archetypal regulatory region occurs in the course of infection in immunosuppressed hosts is consistent with the adaptation hypothesis which has been put forward to explain the divergence of the regulatory regions in PML-derived JC virus isolates.

JC polyomavirus (JCV) is widespread in the human population (15). It causes a rare demyelinating disease of the central nervous system, progressive multifocal leukoencephalopathy (PML). Groups at high risk for PML are patients with underlying diseases which decrease their immunological capacity and those who are immunosuppressed therapeutically or after organ transplantation (15, 17). However, there have been a few documented cases in which PML occurred in patients with no sign of immunological disorders (18).

Among JCV isolates from PML patients there exists a remarkable diversity in the structures of the regulatory regions on the genomes, although the structures of the other regions are apparently conserved (4, 6, 10–13, 16). Dörries (4), who analyzed JCV DNA from the brain and kidney of the same PML patient, suggested that initial infection with one JCV strain was followed by the development of variants differing in regulatory sequences. In a report describing the complete DNA sequence of a PML-derived isolate, Mad1, Frisque et al. (5) argued that the tandem repeat in the regulatory region of Mad1 might reflect an adaptation of the virus for growth in brain tissue. Recently, we molecularly cloned JCV DNAs from the urine of nonimmunosuppressed patients and healthy volunteers and analyzed them for the structures of the regulatory sequences (25). We found that the regulatory sequences of all cloned JCV DNAs were identical to each other, with a few nucleotide substitutions, but were remarkably different from those of PML-derived JCVs. We showed that this regulatory sequence, designated as an archetypal sequence, could have generated various regulatory sequences of PML-derived isolates (25).

Recently, Myers et al. (14) molecularly cloned JCV DNA from the urine of two immunosuppressed patients, one renal transplant patient, and one bone marrow transplant patient. They obtained four JCV DNA clones and found that each of them contained the same regulatory sequences as one of two JCV isolates derived from PML patients. In this study, we examined the structures of the regulatory regions in a number of JCV DNA clones obtained from urine samples of nine renal transplant patients undergoing immunosuppression.

MATERIALS AND METHODS

Patients. Renal transplant recipients studied here are listed in Table 1. All of the patients were Japanese and received transplants at the Department of Urology, Hamamatsu University School of Medicine. Cyclosporine, azathioprine, mizoribin, and prednisolone had been administered daily in various combinations. None of the patients exhibited any sign of PML. Urine specimens were collected from the patients 3 to 86 months after transplantation (Table 1) and were immediately frozen and stored at −80°C.

Extraction of viral DNA from urine. Urine was fractionated into the urinary sediment and supernatant by centrifugation at 1,300 × *g* for 10 min at 4°C. The supernatant was centrifuged at 100,000 × *g* for 3 h at 4°C. The resultant pellet (virion fraction) was resuspended in 10 mM Tris-HCl–10 mM EDTA (pH 7.6) and processed as described previously to extract DNA (25). The urinary sediment (infected-cell fraction) was resuspended in 10 mM Tris-HCl–10 mM EDTA (pH 7.6) and processed as the virion fraction.

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TABLE 1. Renal transplant recipients analyzed

Patient	Gender,[a] age (yr)	Origin of transplated kidney[b]	Time relative to transplant (mo)[c]	Viral DNA detected in urine
NY	M, 21	LD, F, 51	12	JCV
SS	F, 25	LD, F, 49	13	JCV
ES	F, 31	CD, M, 19	66	JCV
WI	M, 31	LD, F, 51	86	JCV
NK	M, 37	LD, M, 57	73	JCV
KA	M, 47	CD, M, 58	25	JCV
SO	M, 57	LD, M, 22	63	JCV
FO	M, 41	LD, M, 66	51	JCV
RN	M, 25	LD, M, 52	15	JCV
MA	M, 31	LD, F, 56	3	BKV
IS	M, 48	LD, M, 46	69	None

[a] M, Male; F, female.
[b] State (LD, living related donors; CD, cadaveric donors), gender (M, male; F, female), and age (in years) of donors. All donors were Japanese.
[c] Time of urine sample collection.

Cloning of viral DNA. DNA from the urine virion fraction was mixed with *Bam*HI-digested, alkaline-phosphatase-treated pUC19 (this plasmid was used as a carrier DNA as well as a vector) and digested with *Bam*HI, which cleaves JCV DNA at a single site. DNAs recovered were ligated with T4 DNA ligase in the presence of ATP and used for *Escherichia coli* DH5α transformation (8). Colonies containing recombinant plasmids were screened by colony hybridization (20) using JCV [^{32}P]DNA as the probe. Plasmids were extracted from small liquid cultures and analyzed with *Sst*I. Large-scale preparation of representative recombinant plasmids with a cleavage pattern unique to those carrying JCV DNA were analyzed by other restriction enzymes, and their regulatory regions were sequenced.

Recombinant DNAs containing JCV (Mad1) DNA (pJC1-4), JCV (MY) DNA (pJC-MY), JCV (CY) DNA (pJC-CY), and BK virus (BKV) (MT-1) DNA (pBK-MT-1) served as reference viral DNAs. pJC1-4 was cloned by Howley et al. (9), and the others were cloned in our laboratory (23, 25). In recombinant plasmids, the viral DNA was linked at the unique *Bam*HI site to pBR322 (pJC1-4), pUC19 (pJC-MY and pJC-CY), or pAT153 (pBK-MT-1).

Amplification by polymerase chain reaction (PCR). To amplify fragments containing the regulatory region of JCV, JC-1L (5′-GCAAAAAAGGGAAAAACAAGGG-3′) and JC-1R (5′-CATCTGCAGCTGGTGACAAGCCAAAACAG-3′) were used as primers. Their locations on the JCV (CY) DNA are indicated in Fig. 2. Primer JC-1R contains a 5′-terminal hexanucleotide, CATCTG, which is not present in the JCV DNA. Addition of this sequence generates a *Pst*I cleavage site, CTGCAG. *Taq* polymerase was obtained from Perkin-Elmer Cetus. Amplification was carried out according to the manufacturer's instructions in 100 μl of a reaction mixture containing sample DNA derived from 3 ml of urine. The reaction mixture was amplified by 25 cycles in a Thermal Sequencer (Iwaki Glass Co., Ltd.). The cycle profile was 94°C for 1.5 min, 55°C for 1.5 min, and 72°C for 2.5 min.

Restriction of viral DNA. Restriction endonucleases were obtained from Toyobo Co., Ltd. (*Bam*HI, *Bgl*II, *Hin*cII, *Hin*dIII, *Nco*I, and *Pvu*II) and Bethesda Research Laboratories, Inc. (*Sst*I [*Sac*I]). Digestion with each enzyme was carried out as recommended by the suppliers. Double digestion with *Bam*HI and *Sst*I was performed at 37°C in 10 mM Tris-HCl (pH 8.0)–100 mM NaCl–7 mM $MgCl_2$–100 μg of bovine serum albumin per ml. Triple digestion with *Hin*dIII, *Pst*I, and *Sst*I was performed at 37°C in 10 mM Tris-HCl (pH 7.5)–60 mM NaCl–9 mM $MgCl_2$–100 μg of bovine serum albumin per ml.

Agarose gel electrophoresis. Digested DNAs were separated on a 0.6 to 1.8% horizontal agarose gel in TAE or TBE buffer (20), depending on the sizes of fragments to be separated. As size references, the *Hin*dIII fragments of lambda phage DNA or the *Hin*fI fragments of plasmid pUC19 were run in parallel.

Blot hybridization. DNAs separated on a gel were transferred to a nitrocellulose filter by a vacuum blotting system (Pharmacia LKB Biotechnology AB). The filter was hybridized with a mixed probe composed of JCV [^{32}P]DNA and BKV [^{32}P]DNA and washed as described previously (24). Autoradiography was carried out with intensifying screens at −50°C for 7 days.

Sequencing. Two *Hin*dIII-*Nco*I fragments, one containing a region from the origin of DNA replication to the start site of the late leader protein (agnoprotein) and one containing a region from the origin of DNA replication to the start site of T antigens, were inserted into M13 mp18 and mp19 between the *Sma*I and *Hin*dIII sites. Single-stranded DNAs purified from recombinant phages were sequenced by chain termination (21). Sequencing was carried out with overlapping clones representing both DNA strands.

Nucleotide sequence accession numbers. The nucleotide sequence accession numbers (EMBL/GenBank/DDBJ accession numbers) are as follows: D90245, JC virus CY/cl1; D90246, JC virus SS/cl131; D90247, JC virus KA/cl28; D90248, JC virus NY/cl70; D90249, JC virus RN/pcr15; and D90250, JC virus FO/pcr37.

RESULTS

Cloning of JCV DNA in urine specimens from renal transplant patients. JCV DNA was cloned from the urine from 8 of 14 renal transplant patients excreting JCV DNA (Table 2). A relatively large number of clones (16 to 33 clones) were obtained from four patients, and a few were obtained from the others. In general, many clones were obtained from urine specimens collected in an earlier posttransplantation period (12 to 25 months), whereas only a small number of clones were obtained from those collected in a later posttransplantation period, except for a specimen from patient SO (Table 2).

We carried out a series of restriction enzyme analyses for representative clones from each patient. Most of the current isolates and the reference clones MY and CY, previously cloned from healthy individuals (25), were identical with regard to *Bgl*II and *Hin*cII restriction fragment patterns, suggesting that all of the current isolates are similar in genome structure to the reference JCVs. The exception is a clone from patient WI, which had missing fragments or a shift of fragment sizes compared with the other clones. These changes could be ascribed to a deletion of a short segment located in the N-terminal region of the VP1 gene (data not shown). The other four clones from WI had the same *Bgl*II and *Hin*cII cleavage patterns as the reference JCV DNAs (data not shown), suggesting that the clones from WI were mostly intact.

Structure of the regulatory region of the cloned JCV DNAs. We first analyzed the regulatory regions of current JCV isolates with *Sst*I. The archetypal regulatory region spanned two *Sst*I fragments, 250 and 230 bp in length (Fig. 1D). The sizes of these two fragments were compared between the

TABLE 2. Summary of analysis of the regulatory regions of cloned JCV DNAs[a]

Patient	Urinary fraction	Viral DNA isolation[b]	No. of clones analyzed	No. of clones containing indicated type of regulatory sequence	
				Archetypal	Rearranged
NY	Virion	d.c.	34 (3)	33 (2)	1 (1)
SS	Virion	d.c.	24 (3)	24 (3)[c]	0 (0)
KA	Virion	d.c.	23 (3)	22 (2)	1 (1)
SO	Virion	d.c.	16 (2)	16 (2)	0 (0)
WI	Virion	d.c.	5 (2)	5 (2)	0 (0)
NK	Virion	d.c.	2 (2)	2 (2)	0 (0)
ES	Virion	d.c.	1 (1)	1 (1)	0 (0)
FO	Virion	d.c.	1 (1)	1 (1)	0 (0)
	Virion	PCR	12 (3)	11 (2)	1 (1)
	Cell	PCR	12 (2)	12 (2)	0 (0)
RN	Virion	PCR	12 (3)	11 (2)	1 (1)
	Cell	PCR	12 (2)	12 (2)	0 (0)
Total			154 (27)	150 (23)	4 (4)

[a] All clones were analyzed with restriction enzymes, and selected clones (numbers indicated in parentheses) were analyzed by sequencing.

[b] d.c., Direct molecular cloning; PCR, PCR amplification followed by molecular cloning.

[c] Includes one clone carrying a change in the noncoding region to the early side of the origin of replication.

current isolates and the reference JCV isolates (MY and CY) by electrophoresis on a 1.8% agarose gel. The fragments of most clones derived from each urine sample were identical with those in the reference clones (Fig. 1 and Table 2), suggesting that the archetypal regulatory region was predominant in all urine samples. However, a few clones (cl70, cl28, and cl131) with a size change in either *Sst*I fragment were found in urine samples from patients NY, and KA, SS, from whom many clones were analyzed (Fig. 1 and Table 2). It is likely that these clones carried a sequence rearrangement in the regulatory region.

For each urine sample, we sequenced two clones (except when only one clone was available) whose regulatory region appeared archetypal by restriction enzyme analysis. The results demonstrated that all clones analyzed contained archetypal regulatory sequences with nucleotide substitutions at nucleotides (nt) 107, 152, 159, and 217 (Tables 2 and 3). The positions of these variable nucleotides are indicated by asterisks in Fig. 2.

Nucleotide substitutions at 107, 159, and 217 were also observed in JCV isolates from nonimmunocompromised individuals (25). From the patterns of these nucleotide substitutions, seven of eight JCV isolates cloned in this study were classified into two subtypes, tentatively designated CY and MY (Table 3). Most (8 of 10) isolates previously cloned from nonimmunocompromised individuals were also grouped into either the MY and CY subtype (25), and therefore these subtypes represent the major ones circulating in Japan.

Furthermore, we sequenced three clones, one (cl70) from

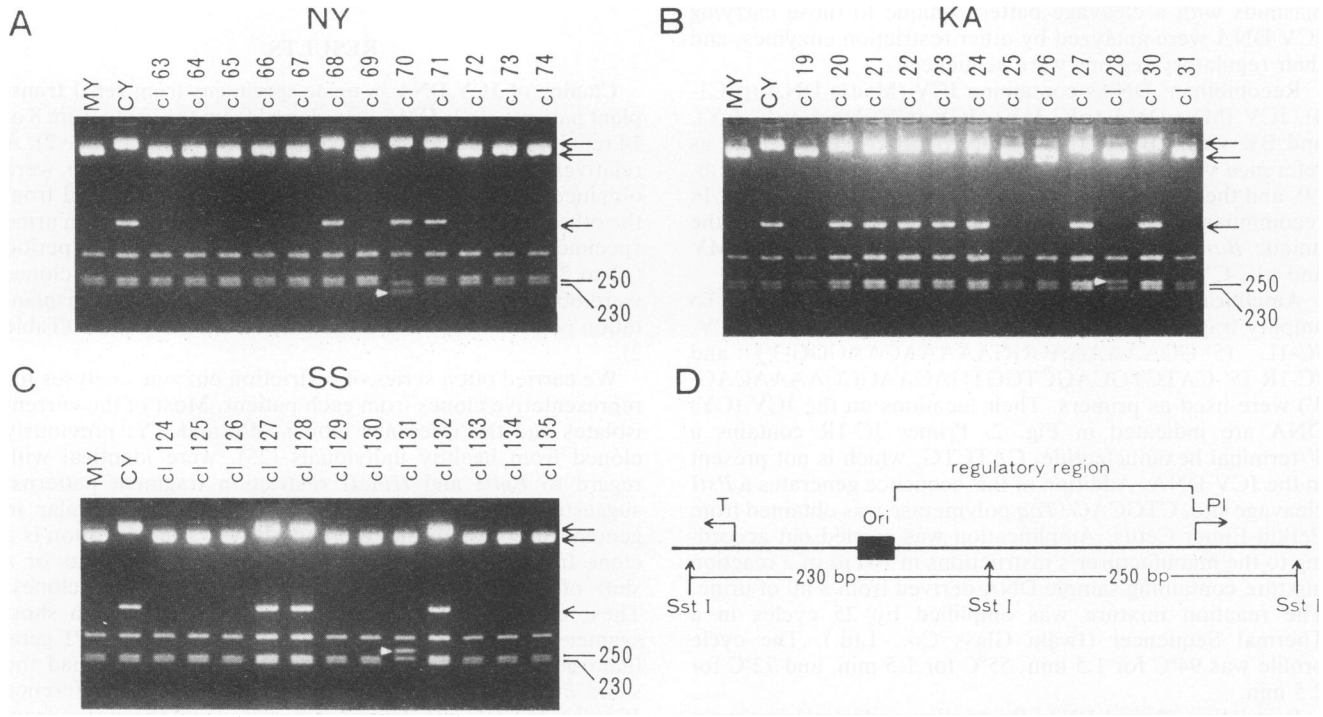

FIG. 1. Restriction enzyme analysis of the regulatory regions cloned from renal transplant patients. Recombinant JCV DNA clones from patients NY (A), KA (B), and SS (C) were digested with restriction enzyme *Sst*I, and fragments generated were electrophoresed on a 1.8% agarose gel. Fragments separated in gels were stained with ethidium bromide and were photographed on a UV-light transilluminator. Sizes of some fragments are indicated in base pairs at the right of each panel. Arrows on the right denote fragments that were produced or not due to the orientation of the integrated viral DNA relative to the vector (the second arrow from the top points to a doublet). An arrowhead in each panel denotes an off-sized fragment. (D) Physical map showing the locations of two *Sst*I fragments. Abbreviations: T, T antigens; Ori, origin of replication; LP1, late leader protein (agnoprotein).

TABLE 3. Nucleotide variations at four positions within the archetypal regulatory regions of JCV isolates obtained from renal transplant patients

Isolate(s)[a]	Nucleotide position[b]				Type[c]
	107	152	159	217	
FO, NY, NK, WI, SS, RN	T	T	C	G	CY
KA, SO	A	T	A	A	MY
ES	T	C	C	G	—[d]

[a] Whole viral DNA was molecularly cloned except for isolate RN, whose regulatory sequence was amplified by PCR and subsequently molecularly cloned.
[b] See Fig. 2.
[c] JCV isolates cloned previously (25) and in this study were classified by the sequence of the regulatory region. A representative isolate is shown for each group.
[d] A new type previously unidentified.

patient NY, one (cl28) from KA, and one (cl131) from SS, which appeared to carry rearrangements in the regulatory regions. Changes in cl70 and cl28 were located in the regulatory region. The change in cl70 was a deletion of two separate segments, while that in cl28 was a duplication of a short stretch of 8 nt (Fig. 2). The change in cl131, involving both deletion and duplication, was not present in the late-side regulatory region but was present in the origin region on the early side (Fig. 2).

Amplification from urine samples of a fragment containing the regulatory region. In the experiments described thus far, the structure of the regulatory region in JCV DNAs cloned from virions excreted in urine were examined. On the other hand, it is known that JCV is also excreted in urine in a cell-associated form (1). Therefore, the structure of its regulatory region also required analysis. For this purpose, we used PCR because of the difficulty of cloning JCV DNA from materials enriched in cellular DNA. DNA was extracted from infected-cell and virion fractions of four patients: two excreting JCV (RN and FO), one excreting BKV (MA), and one excreting neither (IS) (Fig. 3A). From each DNA sample, we attempted to amplify a fragment containing the regulatory region of JCV by using primers JC-1L and JC-1R, whose locations on the JCV DNA are indicated in Fig. 2. After amplification under conditions detailed in Materials and Methods, an aliquot of each reaction mixture was analyzed by electrophoresis on a 1.8% agarose gel (Fig. 3B). Amplification of a 360-bp JCV-specific fragment was clearly seen in the infected-cell as well as in the virion fractions of patients RN and FO.

Structure of the regulatory region of the amplified fragments. We molecularly cloned part of the amplified fragment which contained the whole regulatory region. A segment between an internal *Hin*dIII cleavage site and a *Pst*I cleavage site within primer JC-1R was cloned into plasmid pUC19. Although many clones were obtained, 12 clones for

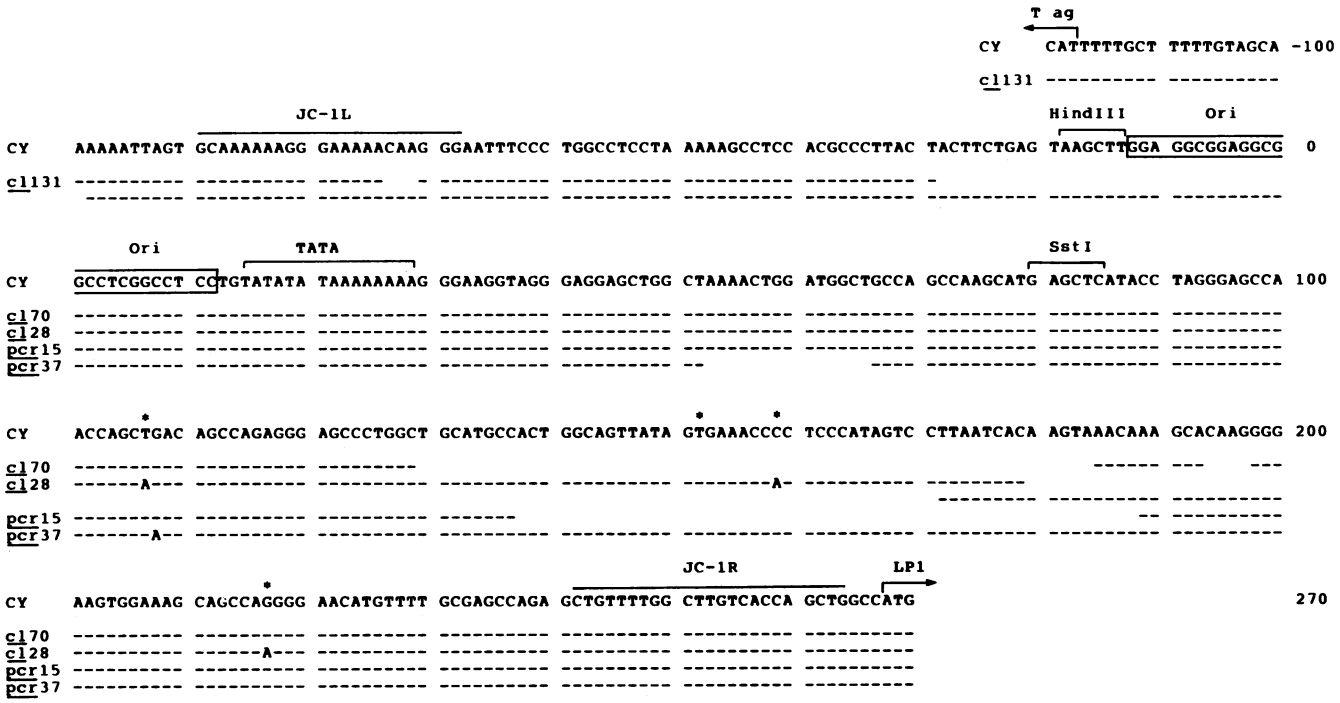

FIG. 2. Structures of rearranged JCV DNAs found in renal transplant patients. The nucleotide sequences from the start site of T antigens (T ag) to the start site of the late leader protein LP1 (agnoprotein) are for isolate CY from a healthy individual (25). Below the CY sequence, the sequence of cl131 (from the start site of T antigens to the origin of replication) and those of cl70, cl28, pcr15, and pcr37 (from the origin of replication to the start site of LP1) are shown. The same nucleotide as in the CY sequence is indicated by a dash. Gaps denote deletions relative to the CY sequence. On reading from left to right, when a repeat is encountered, the linear representation is displaced to the line below and to positions corresponding to the sequence of CY. *Hin*dIII and *Sst*I recognition sequences and locations of primers JC-1L and JC-1R are shown above the sequence. Nucleotides indicated by asterisks are variable among JCV DNA clones isolated in this study (see Table 3). The nucleotide numbering is similar to that of Frisque et al. (5), but because lengths of the JCV DNAs shown here are unknown, nucleotides proceeding backward from the origin of replication are numbered by negative numbers. Abbreviations: Ori, origin of DNA replication (5); TATA, TATA sequence (5).

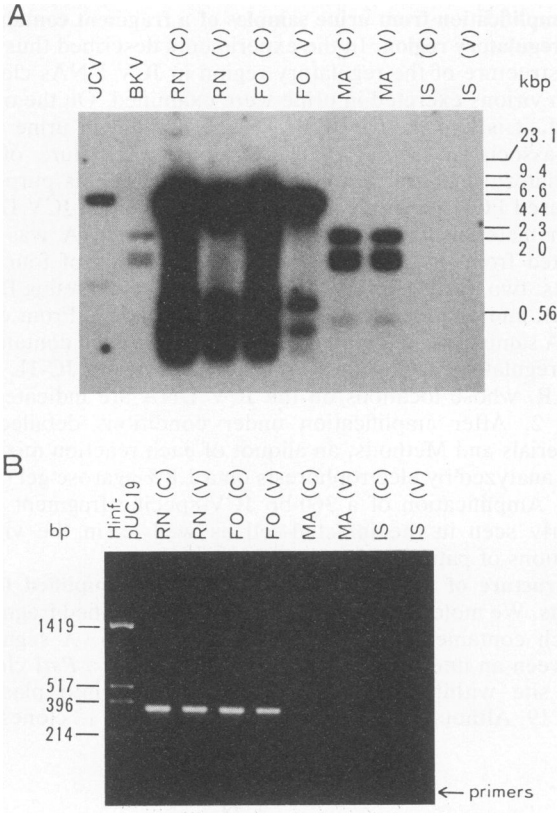

FIG. 3. (A) Detection of JCV and BKV DNAs in the infected-cell and virion fractions of urine samples from the patients indicated. pJC1-4 and pBK-MT-1 containing 6 pg of viral DNAs and sample DNAs derived from 3 ml of urine were digested with a combination of *Bam*HI and *Sst*I, electrophoresed in a 1% agarose gel, and blotted to a nitrocellulose filter. The filter was hybridized with a mixed probe composed of ^{32}P-labeled BKV and JCV DNAs and autoradiographed for 18 h. Positions of various *Hin*dIII fragments of lambda DNA, electrophoresed in parallel and visualized with ethidium bromide, are given on the right as size markers. (B) Amplification of a fragment containing the regulatory region of JCV, using the infected-cell and virion fractions of urine from the patients indicated. DNAs derived from 3 ml of urine were used for amplification. The conditions of amplification were described in Materials and Methods. A 10-μl aliquot of each reaction mixture was analyzed by electrophoresis on a 1.8% agarose gel. *Hin*fI fragments of pUC19, coelectrophoresed on the same gel, are indicated at the left. Abbreviations: C, infected-cell fraction; V, virion fraction.

each sample were analyzed by digestion with a combination of *Hin*dIII, *Sst*I, and *Pst*I and by subsequent agarose gel electrophoresis. The fragment amplified from an authentic JCV (CY) DNA was similarly processed as a reference. Most clones from both urinary fractions gave the same cleavage profile as the reference DNA did. These results suggest that a majority of fragment molecules amplified from infected cells as well as virions contained the archetypal regulatory sequence. The exceptions were two clones, pcr15 and pcr37, from virion fractions of RN and FO, respectively. One of the fragments generated by each of these clones migrated in the gel faster than the corresponding fragment from the reference DNA.

For each fraction of urine derived from patients RN and FO, we sequenced two clones which appeared to have the archetypal regulatory region. In RN as well as FO, the regulatory regions of both clones were of the CY archetype, with T, T, C, and G at nt 107, 152, 159, and 217 (Table 3).

We next sequenced two clones, pcr15 and pcr37, which appeared to be rearranged in the regulatory region according to restriction analysis. The major changes in pcr15 and pcr37 were deletion of different segments located in the regulatory region (Fig. 2). In addition, we detected a nucleotide substitution at nt 108 that was not present in the other clones derived from FO. It is possible that this substitution was introduced during DNA amplification (19).

DISCUSSION

In this study we analyzed the regulatory sequences of JCV DNAs cloned from urine samples of a PML risk group, that is, renal transplant patients on immunosuppressive therapy. We found that clones with the archetypal regulatory sequence were predominant in all urine samples, but a few clones carried regulatory sequences that diverged from the archetypal sequence by deletion or duplication. This finding suggests that sequence rearrangement in the archetypal regulatory region of JCV occurs during infection in immunosuppressed hosts.

Because only 4 of the 154 clones contained altered regulatory sequences, the frequency of rearrangement in the regulatory region appears to be low in the general renal transplant population. However, it is possible that it is high in some renal transplant patients. This is because rearrangement of the regulatory sequence may occur more frequently later after transplantation than earlier, but we analyzed fewer clones from urine samples collected later after transplantation. Furthermore, the sequence of our late-side primer (JC-1R) overlaps with a DNA region which is known to be missing in some PML-derived JCV isolates (e.g., Mad11-Br) (12). Thus, such variants in this study would not have been amplified and therefore would not have gone undetected.

Three of the four clones with rearrangements contained a deletion and one contained a duplication. These rearranged regulatory sequences, together with those of JCV DNAs previously cloned from brain tissues of PML patients (5, 10, 12, 13), are diagrammatically represented in Fig. 4. With the single exception of Mad11-Br, the PML-derived isolates shared some structural features: duplication of domains A (47-bp sequence) and C (9-bp sequence) and deletion of domain B (55-bp sequence) (Fig. 4A and B). Interestingly, one clone (cl70) in this study was lacking a segment overlapping domain B to a great extent. It is possible that the deletion which occurred in cl70 represents the initial stage of adaptation; subsequent duplication of domains A and B may have not yet occurred.

We previously reported that duplication of a 31-bp sequence spanning from nt 201 to 231 occurred in the regulatory region of a JCV DNA clone from the urine of a healthy individual (CY) from which multiple clones were analyzed (25). Therefore, rearrangement of the regulatory region appears not to be limited to immunosuppressed hosts. However, if JCV replicates more actively in immunosuppressed hosts than in normal hosts, the number of rearranged JCV DNA molecules generated would increase in immunosuppressed hosts. This assumption is consistent with the fact that PML occurs more frequently in patients with relative immunological unresponsiveness than in those with normal responsiveness (17).

The present and previous (25) studies showed that deletion and duplication occurred in various sites of the archetypal

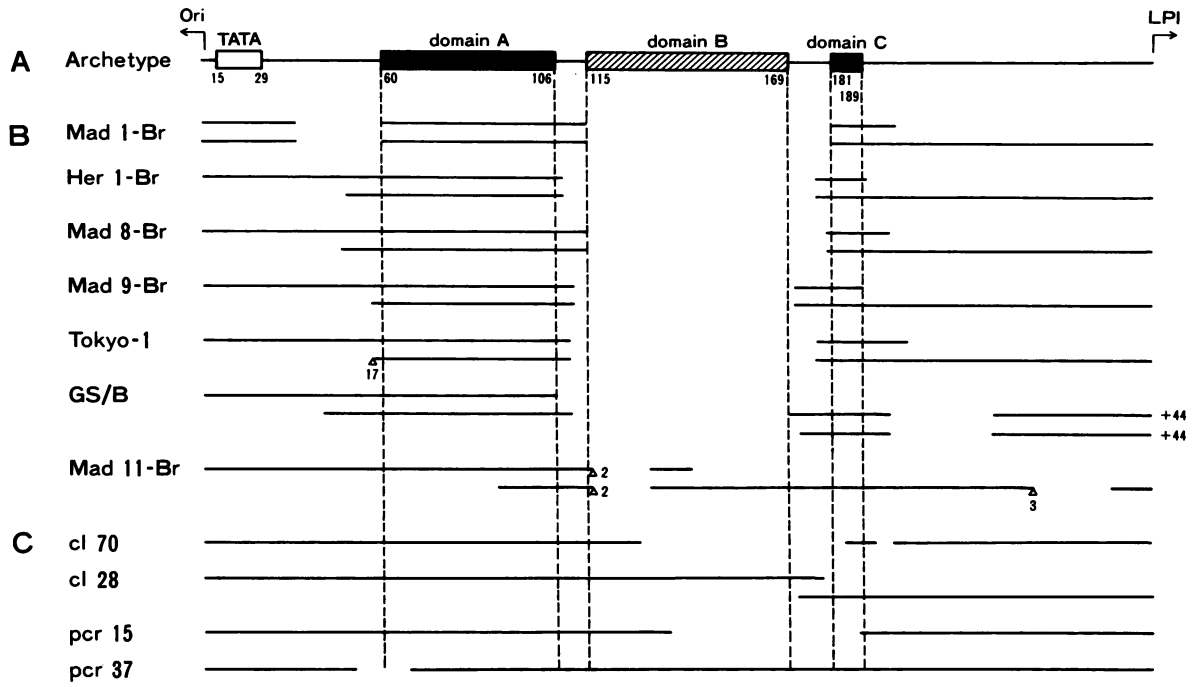

FIG. 4. Diagrammatic representation of regulatory sequences of various PML-derived JCVs and some JCVs from renal transplant patients. (A) Structure of the archetypal regulatory region. TATA indicates TATA sequence (5); domains A and C indicate sequences duplicated in most PML-derived JCVs except Mad11-Br, and domain B indicates a sequence deleted in most PML-derived JCVs except Mad11-Br. Numbers below each box are nucleotide numbers indicating end locations (the nucleotide numbering system is that of Frisque et al. [5]). The origin of DNA replication (Ori) and the start site of the late leader protein (LP1) are shown. See Fig. 2 for the nucleotide sequence of the archetypal regulatory region. (B) Structures of the regulatory regions of JCV DNAs that were directly cloned from brain tissue with PML. Deletions relative to the archetype are shown as gaps. On reading from left to right, when a repeat is encountered, the linear representation is displaced to the line below and to positions corresponding to the sequence of the archetype (25). Numbers at the right of the structure of GS/B indicate that the lines are extended by a length indicated in base pairs. Arrowheads represent the insertion of sequences whose sizes are indicated below in base pairs. The structures of PML-derived isolates are drawn on the basis of the sequence data reported by Frisque et al. (5), Martin et al. (12), Matsuda et al. (13), and Loeber and Dörries (10). (C) Rearranged regulatory sequences in JCV DNA clones and in fragment clones derived from renal transplant patients. cl70 and cl28 (clones containing the whole genome of JCV) were isolated from the virion fractions of patients NY and KA, respectively. pcr15 and pcr37 (clones containing part of the genome) were obtained after PCR amplification from the virion fractions of patients RN and FO, respectively.

regulatory region. Most of these changes may not be advantageous to the growth of JCV in the kidney, an organ probably supporting the growth of JCV (2), and therefore most variants carrying these changes could not readily become a major species in the JCV population in the kidney. Among these variants, some rare ones, arising from both deletion and duplication, may acquire a higher growth capacity in brain but not in kidney. This can explain the generation of PML-type JCVs and is consistent with the adaptation hypothesis which has previously been suggested by others to explain the divergence of PML-derived JCVs (3–5, 10).

The current finding that JCV DNAs carrying the archetypal regulatory sequence are predominant in renal transplant patients contrasts with those by Myers et al. (14). They cloned JCV DNAs from the urine of one renal transplant recipient and one bone marrow recipient, both of whom were receiving immunosuppressive therapy. Each of four JCV DNA clones obtained by them was identical to one of two previously obtained PML-derived JCV isolates, Mad1-TC and Mad8-Br. (The regulatory sequence of Mad1-TC is identical to that of Mad1-Br, which was molecularly cloned directly from brain tissue of the same PML patient [12]. The regulatory sequences of Mad1-Br and Mad8-Br are schematically shown in Fig. 4B.) Some speculations are possible concerning the origin of the PML-type variants isolated by Myers et al. (14). For example, they may have evolved from archetypal strains in the course of infection in the immunosuppressed patients studied. Alternatively, JCV strains similar to Mad1-TC and Mad8-Br, rather than archetypal strains, may be prevalent in some areas of the United States.

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REFERENCES

1. **Arthur, R. R., and K. V. Shah.** 1989. Occurrence and significance of papovaviruses BK and JC in the urine. Prog. Med. Virol. **36**:42–61.
2. **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.
3. **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.
4. **Dörries, K.** 1984. Progressive multifocal leucoencephalopathy: analysis of JC virus DNA from brain and kidney tissue. Virus Res. **1**:25–38.
5. **Frisque, R. J., G. L. Bream, and M. T. Cannella.** 1984. Human polyomavirus JC virus genome. J. Virol. **51**:458–469.
6. **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.
7. **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.
8. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166**:557–580.
9. **Howley, P. M., F. Rentier-Delrue, C. A. Heilman, M.-F. Law, K. Chowdhury, M. A. Israel, and K. K. Takemoto.** 1980. Cloned human polyomavirus JC DNA can transform human amnion cells. J. Virol. **36**:878–882.
10. **Loeber, G., and K. Dörries.** 1988. DNA rearrangement in organ-specific variants of polyomavirus JC strain GS. J. Virol. **62**:1730–1735.
11. **Martin, J. D., and G. C. Foster.** 1984. Multiple JC virus genomes from one patient. J. Gen. Virol. **65**:1405–1411.
12. **Martin, J. D., D. M. King, J. M. Slauch, and R. J. Frisque.** 1985. Differences in regulatory sequences of naturally occurring JC virus variants. J. Virol. **53**:306–311.
13. **Matsuda, M., M. Jona, K. Yasui, and K. Nagashima.** 1987. Genetic characterization of JC virus Tokyo-1 strain, a variant oncogenic in rodents. Virus Res. **7**:159–168.
14. **Myers, C., R. J. Frisque, and R. R. Arthur.** 1989. Direct isolation and characterization of JC virus from urine samples of renal and bone marrow transplant patients. J. Virol. **63**:4445–4449.
15. **Padgett, B. L., and D. L. Walker.** 1976. New human papovaviruses. Prog. Med. Virol. **22**:1–35.
16. **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.
17. **Richardson, E. P., Jr.** 1961. Progressive multifocal leukoencephalopathy. N. Engl. J. Med. **265**:815–823.
18. **Rockwell, D., F. L. Ruben, A. Winkelstein, and H. Mendelow.** 1976. Absence of immune deficiencies in a case of progressive multifocal leukoencephalopathy. Am. J. Med. **61**:433–436.
19. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239**:487–491.
20. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed., book 1. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
21. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
22. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
23. **Sugimoto, C., K. Hara, F. Taguchi, and Y. Yogo.** 1989. Growth efficiency of naturally occurring BK virus variants in vivo and in vitro. J. Virol. **63**:3195–3199.
24. **Yogo, Y., A. Furuno, A. Nozawa, and S. Uchida.** 1981. Organization of viral genome in a T-antigen-negative hamster tumor induced by human papovavirus BK. J. Virol. **38**:556–563.
25. **Yogo, Y., T. Kitamura, C. Sugimoto, T. Ueki, Y. Aso, K. Hara, and F. Taguchi.** 1990. Isolation of a possible archetypal JC virus DNA sequence from nonimmunocompromised individuals. J. Virol. **64**:3139–3143.