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Isolation of a Possible Archetypal JC Virus DNA Sequence from Nonimmunocompromised Individuals

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We molecularly cloned JC polyomavirus DNAs from urine samples of eight nonimmunosuppressed patients and two healthy individuals. The cloned viral DNAs all contained an archetypal regulatory sequence from which various regulatory sequences of JC polyomavirus isolates derived from patients with progressive multifocal leukoencephalopathy could have evolved by deletion and amplification.

JC polyomavirus (JCV) is widespread in the human population (10). While it is not clear whether JCV is associated with any disease in the general population, in immunodeficient or immunosuppressed patients it causes a fatal demyelinating disease of the central nervous system, progressive multifocal leukoencephalopathy (PML) (10). The complete DNA sequence determined for a strain (Mad-1) derived from a brain of a PML patient has shown that the genome of JCV, like that of BK virus (BKV), a closely related human polyomavirus, consists of the early, late, and regulatory regions (2). The structure of the regulatory region has been elucidated for many JCV isolates derived from PML patients, and it has been found that there exists remarkable diversity in the structures of the regulatory regions (1, 3, 6–8, 11). However, it is unknown how this variation was introduced. Here we report that JCV DNAs isolated by molecular cloning from the urine samples of nonimmunosuppressed patients and healthy individuals all contained an archetypal regulatory region from which the various regulatory regions of PML-derived JCV isolates could have evolved.

We have recently found that nonimmunosuppressed, older patients excrete JCV DNA into urine at a high frequency (about 50%) (T. Kitamura, Y. Aso, N. Kuniyoshi, K. Hara, and Y. Yogo, J. Infect. Dis., in press). To clone this JCV DNA, a number of urine samples (about 100 in total) were collected from nonimmunosuppressed, older patients in five hospitals located in and around Tokyo, Japan. In addition, urine samples were collected from several healthy volunteers living in various regions of Tokyo. After removal of urinary sediment, urine samples were subjected to a high-speed centrifugation at 100,000 × *g* for 3 h to pellet virions. From the resultant pellets, DNA was extracted by digestion with proteinase K and subsequent phenol treatment and was screened for JCV DNA by Southern blot hybridization (14). From DNA samples which were found to contain a large amount of JCV DNA, this JCV DNA was cloned at its unique *Bam*HI cleavage site by using plasmid pUC19 and *Escherichia coli* DH5α (Bethesda Research Laboratories, Inc., Gaithersburg, Md.). We obtained full-length JCV DNA clones from eight patients with various diseases and from two volunteers (Table 1). From three individuals, many clones were obtained, while only one or a few were obtained from the others.

First we carried out restriction endonuclease analysis to make a comparison within the isolates of the present study as well as between those of the present study and those derived from PML patients. PML-derived isolates used were Mad-1 [pJC(1-4)] (4) and Tokyo-1 (pJCT-Br) (8), which were molecularly cloned from the brains of PML patients in the United States and in Japan, respectively. pJC(1-4) and pJCT-Br were obtained from Japanese Cancer Research Resources Bank and from K. Yasui, respectively. Since, in pJCT-Br, JCV DNA was linked to a vector at the *Eco*RI site, the JCV DNA was excised from the vector and a full-length JCV DNA was recloned at the *Bam*HI site. JCV DNAs were recovered from representative clones obtained in the present study and from reference clones by *Bam*HI digestion and were digested with *Hin*cII, *Sst*I (*Sac*I), *Bgl*II, or *Pvu*II. Fragments generated were separated by agarose gel electrophoresis (Fig. 1). The results can be summarized as follows.

(i) In an analysis in which *Hin*cII, *Sst*I (*Sac*I), or *Bgl*II was used, the present isolates were very similar to one another and to Tokyo-1 but were clearly distinguished from Mad-1 (Fig. 1A through C). This finding suggests that the present isolates and Tokyo-1 represent a subtype of JCV prevalent in Japan. The presence in Japan of a subtype of JCV different from those in the United States and in Germany was previously suggested by Matsuda et al. (8) on the basis of restriction analysis of Tokyo-1.

(ii) *Sst*I digestion revealed that a common difference exists between the present isolates and the PML-derived isolates (Tokyo-1 and Mad-1). After *Sst*I digestion, each of the present isolates generated two fragments of a similar size (about 260 base pairs [bp]) (Fig. 1B). One of these fragments was also generated by Tokyo-1 and not by Mad-1, suggesting that this fragment is common to the Japanese subtype of JCV. Tokyo-1, however, did not generate the other 260-bp fragment but instead gave rise to two fragments of 210 and 90 bp (Fig. 1B). Fragments similar to these two were also produced by Mad-1 (Fig. 1B). Since the 90-bp fragment is generated because an *Sst*I site is present in each of the tandem repeats in Mad-1 and Tokyo-1 (2, 8), the absence of this fragment (or a fragment of similar size) suggests that each of the present isolates does not retain tandem repeats.

The sum of the sizes of the 210- and 90-bp fragments was

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TABLE 1. Nucleotides at seven variable positions within the regulatory region of JCV

Isolate of JCV[a]	Condition of donor, source of specimen	Nucleotide position[b]						
		107	108	109	143	159	217	242
CY	Healthy, urine	T	G	A	C	C	G	C
MY	Healthy, urine	A	G	A	C	A	A	C
UA	Urologic disease,[c] urine	T	G	G	C	C	G	C
SI	Urologic disease,[c] urine	T	G	A	C	C	G	C
YI	Urologic disease,[c] urine	T	G	A	C	A	A	C
HS	Urologic disease,[c] urine	A	G	A	C	A	A	C
ST	Cerebrovascular disease, urine	A	G	A	C	A	A	C
KF	Cerebrovascular disease, urine	A	G	A	C	A	A	C
HA	Neurologic disease, urine	A	G	A	C	A	A	C
MS	Malignancy, urine	T	G	A	C	C	G	C
GS/K	PML, kidney	T	G	A	—[d]	C	A	A
Mad-1	PML, brain	T	A	A	C	—	A	C
Her-1	PML, brain	T	G	A	C	—	A	C
Tokyo-1	PML, brain	A	G	A	C	—	A	C

[a] CY through MS were molecularly cloned and analyzed in this study. GS/K (5), Mad-1 (2), Her-1 (7), and Tokyo-1 (8) were from the references indicated.
[b] See Fig. 2.
[c] Urologic disease here indicates acute cystitis or benign prostate hypertrophy.
[d] —, Absence of a corresponding nucleotide.

not equal to the size of the 260-bp fragment. Since the largest part of the regulatory sequence is included in the 210- and 90-bp fragments (2, 8) or in the 260-bp fragment (data not shown), the size discrepancy described above suggests that the present isolates and the PML-derived isolates differ in the length of the regulatory region. The size difference of fragments containing the regulatory region was also observed between the present isolates and the PML-derived isolates when other restriction enzymes such as *Hae*III and *Nco*I were used (data not shown).

(iii) The present isolates could be divided into two groups with respect to the presence or absence of a *Pvu*II cleavage site (Fig. 1D). After digestion with *Pvu*II, one group (KF, MY, HA, HS, and ST) gave rise to a 930-bp fragment not generated by the other (CY, UA, SI, YI, and MS). Since the latter generated instead two shorter fragments of 800 and 130 bp, isolates of this group must contain an extra cleavage site within the 930-bp fragment. In addition, one isolate (YI) apparently carried an additional *Pvu*II site, since one *Pvu*II fragment of this isolate (indicated by an arrowhead) was

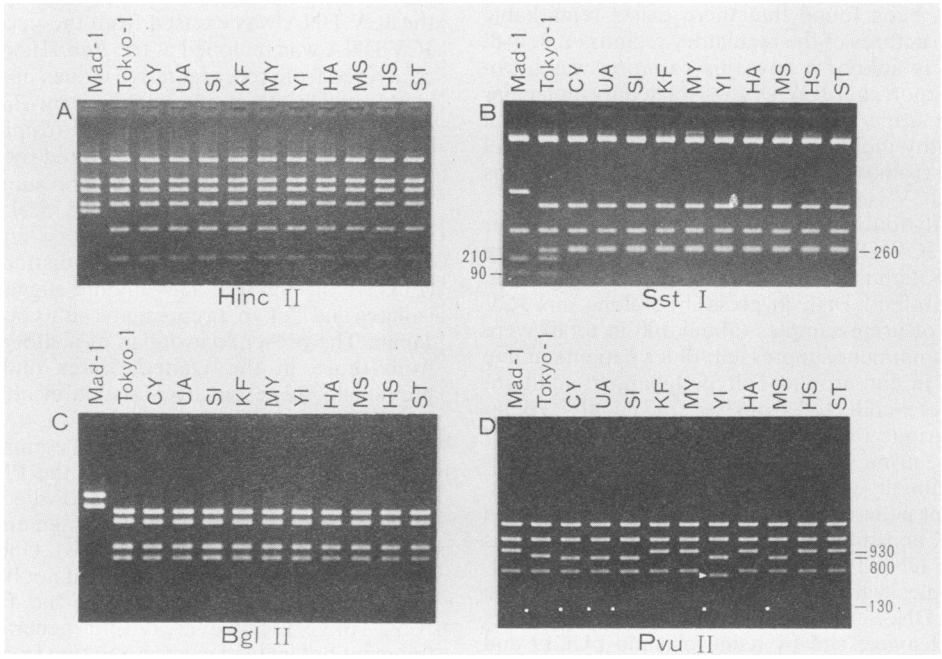

FIG. 1. Restriction of enzyme analysis of JCV DNAs cloned from urine samples of nonimmunosuppressed or healthy individuals. Ten representative JCV DNAs from nonimmunosuppressed or healthy individuals (listed in Table 1) and two PML-derived JCV DNAs (Mad-1 and Tokyo-1) were recovered from recombinant DNAs and were digested with restriction enzymes *Hinc*II (A), *Sst*I (B), *Bgl*I (C), and *Pvu*II (D). Fragments generated were electrophoresed on either a 1.8% (*Hinc*II and *Sst*I) or 1% agarose gel (*Bgl*II and *Pvu*II). Fragments separated in gels were stained with ethidium bromide and were photographed on a UV light transilluminator. Sizes of some *Sst*I and *Pvu*II fragments are indicated in base pairs on the left (for Mad-1 and Tokyo-1) or the right (for the present isolates) of panels B and D. Note that two fragments of 260 bp comigrated on lanes CY through ST of panel B. Fragments of approximately 130 bp are indicated by dots on panel D. An arrowhead in panel D denotes a fragment unique to isolate YI.

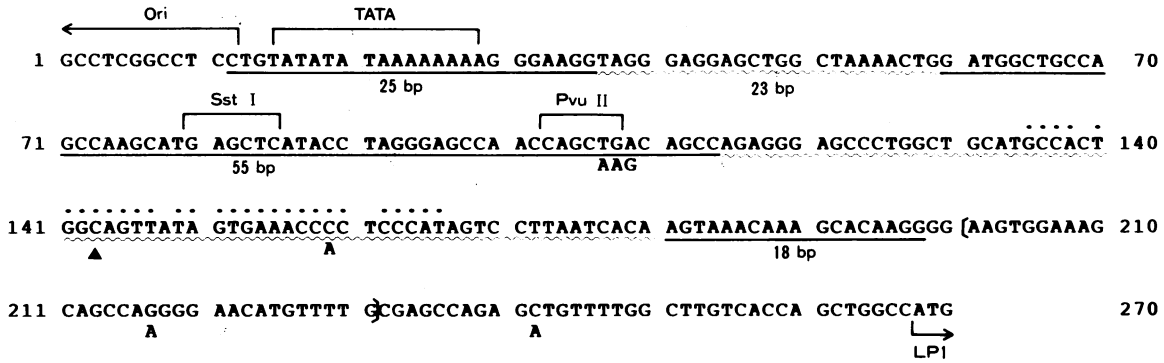

FIG. 2. Structure of the regulatory region of JCV DNA cloned from a healthy individual (CY). A recombinant JCV DNA-derived *Hin*dIII-*Nco*I fragment (about 290 bp) containing both the origin of DNA replication and the regulatory region was inserted into M13 mp18 and mp19 between the *Sma*I and *Hin*dIII sites. Single-stranded DNA purified from recombinant bacteriophage was sequenced by the chain termination method (13). The nucleotide sequence shown is from the origin of DNA replication to the start site of the late leader protein (agnoprotein). Alternative nucleotides found in other JCV DNA clones analyzed in this study and in JCV DNAs reported previously (2, 5, 7, 8) are indicated below the sequence of CY (the absence of a corresponding nucleotide is indicated by an arrowhead) (see Table 1). The 25-, 55-, and 18-bp sequences present in the 98-bp repeated sequences of Mad-1 (2) are shown by straight lines, and 23- and 66-bp sequences not present in Mad-1 are shown by wavy lines. A region bounded by parenthesis is duplicated in a JCV DNA clone of isolate CY. *Sst*I and *Pvu*II recognition sequences are indicated above the sequence. The nucleotide numbering system is that of Frisque et al. (2). Abbreviations: Ori, origin of DNA replication (2); TATA, TATA sequence (2); LP1, start site of the late leader protein (2). ...., Homology between JCV (CY) DNA and BKV (MT-1) DNA (15).

shorter than the corresponding fragments of the other isolates. These results suggest that the Japanese subtype of JCV can be further classified into a few groups. Sequence analysis described below showed that one of the variations concerning *Pvu*II cleavage sites could be explained by a nucleotide variation, A or T, at nucleotide (nt) 107 (Fig. 2 and Table 1). The presence of an extra *Pvu*II site was also detected in one of the two PML-derived isolates (Tokyo-1) (Fig. 1D); this *Pvu*II site was introduced by insertion of a 17-bp sequence (8).

We next analyzed the sequences of the regulatory regions of the present isolates. The sequence was determined for at least one clone per individual, and in three cases, five to six clones were examined for the homogeneity of the regulatory region among JCV DNAs derived from the same individual. The basic structure of the regulatory region was identical among clones derived from all individuals, with a few base mismatches. The regulatory sequence of a clone from a healthy individual (CY) is shown in Fig. 2 (a diagrammatic representation is also given at the top of Fig. 3), and nucleotides at variable positions in each JCV DNA clone are in Table 1. In each case where multiple clones from the same individual were compared, the structure of the regulatory region was identical among clones examined, with one exception in which one of six clones analyzed carried a duplication of a short segment, indicated by brackets in Fig. 2. The regulatory region of the cloned JCV DNAs lacked any repetition of a sequence of significant length. It contained 23 and 66-bp sequences, which were inserted into the 98-bp sequence present in a tandem repeat in Mad-1 (2). Consequently, the 98-bp sequence was split into three portions of 25, 55, and 18 bp in size. The 23-bp sequence has been previously identified in a majority of PML-derived variants (7, 8), although it was absent in a few, including Mad-1 (2, 7). A 32-bp stretch within the 66-bp sequence is highly homologous to a sequence present in a corresponding region of BKV. This homology was previously identified in a PML-derived isolate (Mad-11Br) (7). For convenience, we designate the regulatory region depicted in Fig. 2 as the archetypal sequence.

The archetypal regulatory sequence is remarkably different from most of the regulatory sequences reported previously (2, 7, 8). However, it is almost identical to that of an isolate (designated as GS/K) which was molecularly cloned directly from the kidney of a PML patient in Germany (5). In a comparison of the CY archetype (Fig. 2) and the GS/K regulatory sequences (5), we found that there are three nucleotide deferences between the CY archetype (Fig. 2) and GS/K (5); in GS/K, the C at nt 143 is missing and nt 217 and 242 are substituted for As. The nucleotide change at nt 217 frequently occurred in the present isolates (Table 1), but that at nt 242 is unique to GS/K (Table 1). Furthermore, the deletion of nt 143 seems to be unique to GS/K, since the C at nt 143 is present in each of the present isolates as well as two PML-derived isolates, Mad-7D and Mad-11Br, containing partial copies of the 66-bp block (7). These features of GS/K may represent those of a subtype of JCV in Germany. Alternatively, they had been introduced into an archetypal JCV in the course of an infection.

As described above, the present JCV isolates molecularly cloned from nonimmunosuppressed patients and from healthy individuals regularly contained the archetypal regulatory region. The unique structure of the archetypal regulatory region was highly conserved among the JCV DNA clones examined, presenting a sharp contrast to the hypervariability of the regulatory region in PML-derived JCV isolates. Furthermore, the archetypal regulatory region contained a sequence homologous to that of BKV. JCV with the archetypal regulatory region may represent the JCV circulating in the human population.

In Fig. 3, the archetypal regulatory region is diagrammatically represented at the top and those of various PML-derived isolates (2, 5, 7, 8) are given below, showing deletions relative to the archetype as gaps and repeats as parallel straight lines. An analogous representation was previously used by Rubinstein et al. (12) to compare various regulatory sequences of BKV. From Fig. 3, it is evident that the regulatory region of each PML-derived isolate could have evolved from the archetype as a consequence of deletion and amplification. There is no simple way in which

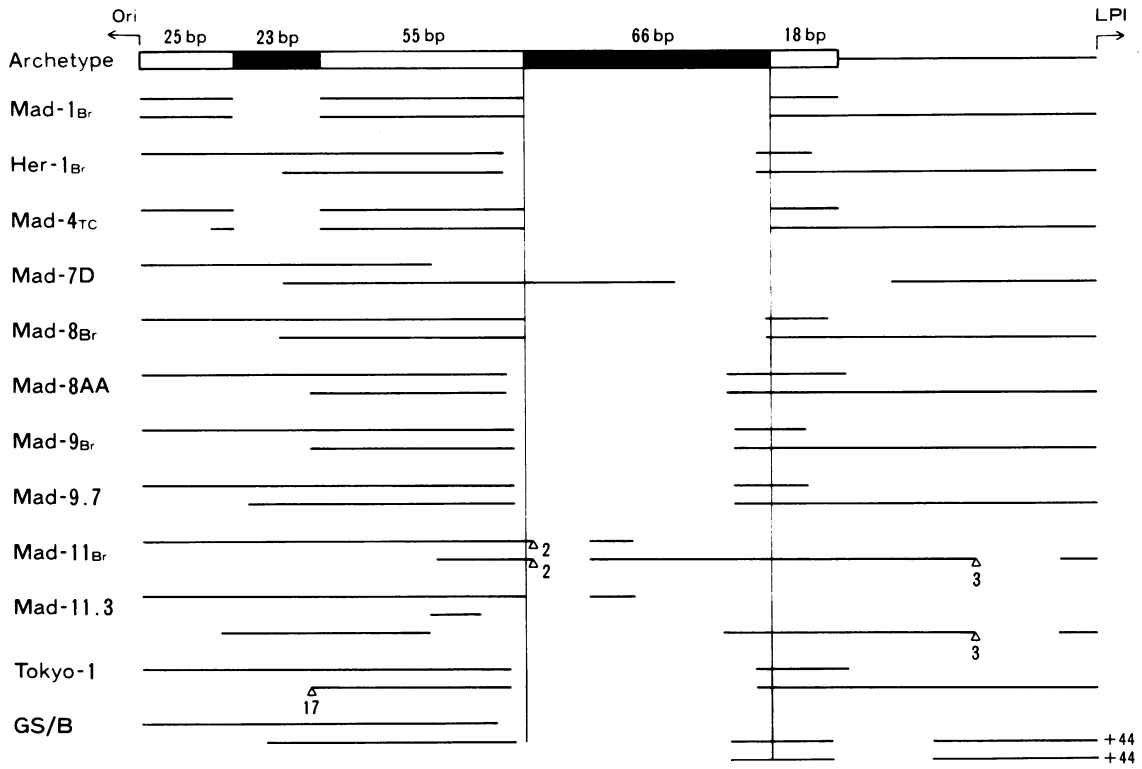

FIG. 3. Diagrammatic representation of regulatory sequences of an archetypal JCV isolate and various PML-derived isolates. The structure of the archetypal regulatory region is shown at the top. Open boxes lettered 25, 55, and 18 bp indicate sequences present in the 98-bp repeated sequences in strain Mad-1 (2), and closed boxes lettered 23 and 66 bp indicate sequences not present in Mad-1. The origin of DNA replication (Ori) and the start site of the late leader protein (LP1) are shown. The structures of the regulatory regions of PML-derived JCV strains are shown below to represent deletions relative to the archetype 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 (12). Numbers at the right-hand ends of the last two lines indicate that the lines are extended by an extra 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. (2), Martin et al. (7), Matsuda et al. (8), and Loeber and Dörries (5).

various structures of PML-derived isolates could have evolved from any of the arrangements other than the archetype. For example, although the lengths and locations of deletions are variable among different PML-derived isolates, each contains identical deletions, with exactly the same 5′ and 3′ endpoints (Fig. 3). This can be simply explained by hypothesizing that in the archetypal sequence, deletion occurred first and the segment carrying the deletion was subsequently duplicated.

If the altered regulatory regions of PML-derived isolates have evolved from the archetypal regulatory region, what host conditions could have induced such changes? Recently, Myers et al. (9) have reported that each of four JCV DNAs cloned from the urine of two transplant patients undergoing immunosuppressive therapy carried a regulatory sequence identical to one of two PML-derived isolates, Mad-1TC and Mad-8Br (7). Although their finding suggests that immunosuppression could be a factor involved in the induction of changes in the regulatory region, examination of JCV isolates derived from a larger number of immunosuppressed patients is required to draw a definite conclusion.

It will be of particular interest to elucidate what effect was produced on the biological properties of JCV by the alteration of the regulatory region from an archetype to a variant structure found in PML-derived isolates. A study on this line is now in progress in our laboratories.

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