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Persistent JC Virus (JCV) Infection Is Demonstrated by Continuous Shedding of the Same JCV Strains

TADAICHI KITAMURA,[1] CHIE SUGIMOTO,[2] ATSUSHI KATO,[3] HIDEKI EBIHARA,[4] MAKOTO SUZUKI,[1] FUMIAKI TAGUCHI,[4] KAZUKI KAWABE,[3] AND YOSHIAKI YOGO[2]*

Department of Urology, Branch Hospital, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 112,[1] Department of Viral Infection, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108,[2] Department of Urology, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113,[3] and Department of Microbiology, School of Allied Health Sciences, Kitasato University, Sagamihara, Kanagawa 228,[4] Japan

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The polyomavirus JC virus (JCV), the causative agent of progressive multifocal leukoencephalopathy, is ubiquitous in the human population, infecting children asymptomatically. JCV is often detected in normal renal tissue and in the urine of healthy individuals. We demonstrate that renal JCV represents that which persists after primary infection.

JC polyomavirus (JCV) was first isolated from the brain of a patient with progressive multifocal leukoencephalopathy (PML) (21). This virus, however, is ubiquitous in humans. Seroepidemiological studies indicated that most individuals are infected with JCV asymptomatically in childhood (19, 20). JCV occurs in the normal renal tissue of most adults (4, 26). Renal JCV is not latent but replicates to excrete progeny in the urine (13, 14, 26).

The genomes of renal as well as urinary JCVs are characterized by a unique regulatory sequence designated as the archetype (26, 30). It has been proposed that archetypal JCV gives rise to JCV variants associated with PML (PML-type JCVs) by sequence rearrangements in the regulatory region (see reference 24 for a review). Moreover, it is likely that the archetypal JCV represents the JCV circulating in the human population (30). In fact, JCV with the archetypal regulatory sequence regularly occurs in urine samples collected from various human populations (1, 2, 8, 10, 16, 29, 30).

JCV DNAs throughout the world can be classified into types A to C according to restriction fragment length polymorphism and sequence variation (10, 29). Type A is prevalent only in Europe, type B is found mainly in Asia and Africa, and type C is localized to part of Africa (10, 29). Type B contains several subtypes. For example, two prevalent subtypes (CY and MY) in Japan belong to type B (15).

It is generally believed that in immunocompetent hosts, following primary infection, JCV indefinitely persists in renal tissue without obvious symptoms (24). One piece of evidence that renal JCV represents persistent infection is the detection of JCV DNA in urine samples sequentially collected from the same individuals (13). However, it is conceivable that after the first JCV infection, the kidney is infected again with JCV from other infected hosts. Thus, renal JCV may represent the virus produced by repeated acute infections. This issue was addressed in this study.

Nineteen Japanese patients participated in this study, as shown in Table 1. All of them were unrelated and resided in or around Tokyo. Paired urine samples were collected from each patient at intervals of 4.7 to 6.8 years. Urine samples in the first set were positive for JCV DNA (13, 14). Samples in the second set were collected for this study and analyzed by PCR for the presence of JCV DNA. All of these urine samples were positive for JCV DNA (data not shown). Thus, we obtained 19 paired urine samples which were collected from the same patients at intervals of approximately 5 to 7 years (Table 1).

A 610-bp JCV DNA region (IG region) (nucleotides 2131 to 2740 [the nucleotide numbering system is that of Frisque et al. {9}]) (3) was amplified from the urinary DNAs by PCR using KOD polymerase with proofreading activity (Toyobo Co. Ltd., Osaka, Japan) (15). Amplified fragments were cloned into pUC19, and two clones were sequenced for each DNA sample as described previously (15). Usually they were identical, and we therefore considered them to be the consensus IG sequence. However, in a few urine samples, the two sequences differed by a single nucleotide. We therefore sequenced one more clone. The third sequence was always identical with one of the two sequences previously determined, so we considered the consistent one to be the consensus IG sequence occurring in the urine sample.

We used the consensus IG sequences to identify JCV strains. Without exception, we detected the same JCV strains from paired urine samples collected from the same patients. (The JCV strains detected from individual patients are shown in Table 1.) Thus, we concluded that the renal JCV represents persistent infection. The possibility that the renal JCV is produced by repeated infection with JCVs transmitted from other hosts can be ruled out.

We next examined whether six foreigners staying in Japan for 2 to 10 years carried the JCV strains prevalent in the Japanese population. These foreigners included two from the United Kingdom and one each from the Czech Republic, the Slovak Republic, Morocco, and the People's Republic of China (Table 2). Their IG sequences indicated that the JCV subtypes differed from those in Japan and were designated X-01 to X-06 (Table 2).

We constructed a neighbor-joining phylogenetic tree (23) for all the IG sequences described above, using the reported reference sequences (Fig. 1). According to the phylogenetic tree, three of the JCV strains (X-01, X-03, and X-05) detected from the foreigners were found to be type A, which is unique to Europe and the United States (2, 3, 10, 29). The other three strains (X-02, X-04, and X-06) belonged to type B but were located in subtypes that are distinguished from those (CY and

* Corresponding author. Mailing address: Department of Viral Infection, the Institute of Medical Science, the University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo 108, Japan. Phone: (81)-3-5449-5287. Fax: (81)-3-5449-5409. E-mail: yogo@ims.u-tokyo.ac.jp.

TABLE 1. Native Japanese patients analyzed

Urine donor (reference)[a]	Gender/age (yr)[b]	Condition[c]	Period (yr) between samplings	JCV strain[d]
P1 (13)	M/8	Urological d.	6.3	M-11
P2 (14)	F/10	Urological d.	4.8	C-11
P3 (14)	F/32	Urological d.	4.9	C-12
P4 (14)	F/32	Urological d.	5.8	C-13
P5 (14)	F/37	Urological d.	5.9	M-12
P6 (14)	M/43	Healthy	5.6	C-14
P7 (14)	F/44	Healthy	4.7	C-15
P8 (13)	F/54	Urological d.	6.8	C-13
P9 (14)	M/67	Urological d.	6.0	C-14
P10 (14)	F/68	Cerebrovascular d.	6.1	C-16
P11 (13)	M/70	Urological d.	5.6	M-12
P12 (13)	F/70	Cerebrovascular d.	6.2	C-17
P13 (13)	M/70	Malignancy	6.2	C-18
P14 (14)	M/70	Cerebrovascular d.	6.2	M-13
P15 (14)	F/72	Cerebrovascular d.	6.0	C-11
P16 (14)	F/72	Malignancy	6.4	C-19
P17 (13)	M/73	Malignancy	6.5	C-20
P18 (13)	M/73	Cerebrovascular d.	6.2	M-12
P19 (14)	F/77	Cerebrovascular d.	6.0	M-14

[a] References in which the collection of the first urine samples is reported are indicated within parentheses.
[b] Age at the time of the first sampling. M, male; F, female.
[c] d., disease.
[d] A JCV strain belonging to subtype CY is designated C-*N*, and that belonging to subtype MY is designated M-*N*, where *N* indicates a double-digit number (15).

MY) unique to the Japanese population. These JCVs (X-1 to -6) probably infected the foreigners during childhood in their home countries and have persisted in their kidneys since then. Thus, the finding that foreigners staying in Japan carried JCV subtypes different from those prevalent in the Japanese population further supported the above-mentioned notion that renal JCV represents persistent infection.

The persistence of JCV in hosts very likely involves the following sequences of events: entry of JCV into the body, multiplication at the site of entry, viremia with transport of JCV to the target organ, and viral persistence in the target organ. However, details of each event remain to be elucidated. For example, neither has the entry site of JCV been identified nor has the source of infection been demonstrated, although the urinary JCV is the most likely candidate for the latter. Nevertheless, this study has established that the target of JCV persistence is the renal tissue.

In normal hosts, JCV may also exist in sites other than renal tissue. JCV has been recovered from lymphocytes of individuals with and without PML (5, 11, 17, 27) and from the normal human brain (6, 18, 22, 28). Two reports have described the regulatory sequences of the recovered JCV DNAs (27, 28).

TABLE 2. Foreigners studied

Foreigner	Gender/age (yr)[a]	Birthplace	Stay in Japan (yr)	JCV isolate
F1	M/55	United Kingdom	5	X-01
F2	M/38	Slovak Republic	2	X-02
F3	M/34	Morocco	10	X-03
F4	M/23	United Kingdom	5	X-04
F5	M/35	Czech Republic	5	X-05
F6	M/36	People's Republic of China	5	X-06

[a] Age at the time of sampling. M, male.

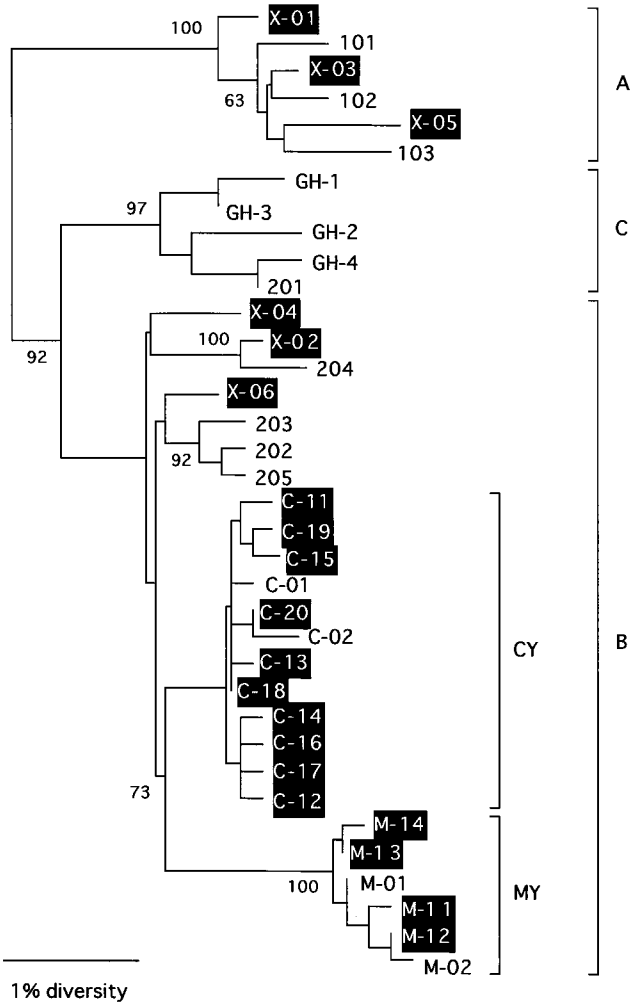

FIG. 1. Neighbor-joining tree relating JCV strains in foreigners who have stayed in Japan for 2 to 10 years with those in Japanese patients. The IG regions (3) of JCV strains from foreigners and Japanese patients were PCR amplified directly from urinary DNAs. The amplified fragments were cloned into pUC19, and purified recombinant plasmids were sequenced (nucleotide sequence accession nos. D88591 to D88610). A neighbor-joining phylogenetic tree (23) was constructed from the determined DNA sequences by using the Clustal W program (25). Divergence was estimated by the two-parameter method (12). The reported IG sequences of isolates 101 through 103, 201 through 205 (3), C-01, C-02, M-01, M-02 (15), and GH-1 through -4 (10) were used as references. Subtypes (CY and MY) and types (A to C) (10) are indicated to the right of the tree. The bootstrap values (7) are given as percentages.

According to these sequences, the JCV DNAs contain PML-type rearranged regulatory regions (30), in sharp contrast to the renal JCV with the archetypal regulatory region. Since the JCVs in peripheral blood lymphocytes as well as in normal brain tissue had PML-type regulatory regions, they may not represent JCVs circulating in the human population.

JCV has been believed to persist in a latent form in the kidney (24). However, the fact that immunocompetent hosts frequently excrete JCV in urine (13, 14) indicates that renal JCV is not latent but replicates to generate progeny in urine. Furthermore, results of the present study exclude the possibility that the renal JCV is produced by repeated infection with JCVs transmitted from other hosts. Thus, JCV persistence in the kidney is characterized by continuous viral replication, causing continuous virus shedding. This would be required for

JCV to be transmitted among humans, provided that JCV infection is very inefficient.

Nucleotide sequence accession number. The DNA sequence data reported in this work have been deposited in the GSDB, DDBJ, EMBL, and NCBI nucleotide sequence databases under accession numbers D88591 to D88610.

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