NKT cells. Although the precise mechanism remains to be elucidated, such increase of γδ T cells in the infected lungs seemed to take place in a different manner from that in NK and NKT cells. Accumulation of NK and NKT cells in lungs after cryptococcal infection was markedly reduced in MCP-1-KO mice, while such reduction was not found in γδ T cells. At present, the precise mechanism of γδ T cell recruitment remains to be clarified.

Interestingly, clearance of *C. neoformans* in lungs was enhanced in mice receiving a manipulation that deletes γδ T cells by administration of specific antibody or targeted disruption of Cδ gene. Such increased host defense was associated with the promoted differentiation of Th1 cells and increased production of IFN-γ. These observations suggest the down-regulatory role of γδ T cells in the host defense to cryptococcal infection. This is in a sharp contrast to the role of NKT cells, which significantly contribute to the development of Th1-type immune response and host resistance to this infection (45). Earlier investigations reported anti-inflammatory γδ T cells that produced Th2 cytokines and TGF-β (97,98). These observations suggest that these cytokines mediate the down-regulatory effect observed in our study. This speculation was supported by our recent data showing the reduced production of TGF-β in the lungs of Cδ-KO mice totally lacking γδ T cells at earlier phase of cryptococcal infection, although the synthesis of Th2 cytokines, IL-4 and IL-10, was not much different from control mice. In this regard, TGF-β is known to suppress the host defense to infectious pathogens (99-102). Furthermore, other investigations revealed that γδ T cells down-regulate the host defense against infection caused by *L. monocytogenes*, *S. choleraesuis* and *Candida albicans* (92-94). Thus, our study suggests that γδ T cells may play down-regulatory roles in the host defense to pulmonary infection with *C. neoformans*.

5. Conclusions

Recently, the role of innate immunity in host defense to infectious pathogens has attracted much attention by many investigators according to the biological significance. In our series of studies on cryptococcal infection, the contribution of NKT and γδ T cells has been unveiled. Contrast roles of NKT and γδ T cells raise a possibility that these innate immune lymphocytes may co-regulate the Th1-mediated response for induction of the moderate host defense, as indicated in Fig. 1. γδ T cells may act to keep the balance of Th1-Th2 responses in a proper manner by suppressing the exaggerated Th1 response caused by NKT cells. In pulmonary infection with *C. neoformans*, number of both NKT and γδ T cells in the paratracheal lymph nodes increases in parallel with that of DCs (our unpublished data), which could be consistent with the above hypothesis. Interestingly, in toxoplasmal infection, γδ T cells appear to play a protective role in the host defense through promoting Th1-mediated immune response, while NKT cells are likely to suppress these responses (91). This is in a sharp contrast to the findings in cryptococcal infection. Thus, NKT and γδ T cells are suggested to participate in the regulation of host defense to infection by bridging from innate to antigen-specific acquired immune responses.

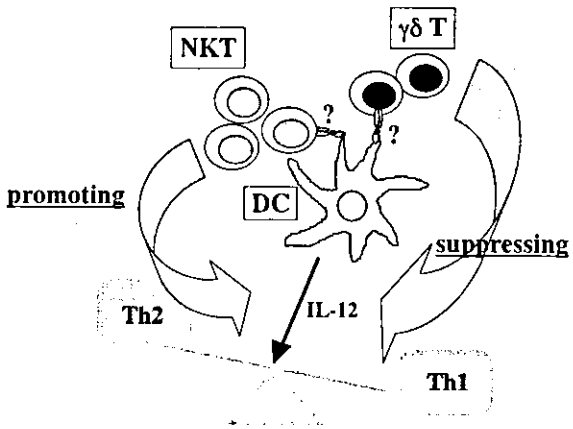

Fig. 1. Regulation of the host defense to cryptococcal infection by NKT and γδ T cells.
Host defense to cryptococcal infection is critically regulated by Th1-Th2 cytokine balance. The predominant synthesis of Th1 cytokines over Th2 protects mice from infection, whereas infection is exacerbated under a Th2-dominant condition. NKT cells regulate this balance to promote the host protection, whereas γδ T cells counter-regulate this process. Thus, these innate immune lymphocytes may act to keep the host defense in a proper manner, although the mechanism of their activation remains to be elucidated.

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Unambiguous identification of *JC polyomavirus* strains transmitted from parents to children

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Summary. *JC polyomavirus* (JCV), the etiological agent of progressive multifocal leukoencephalopathy, is ubiquitous in humans, infecting children asymptomatically, then persisting in renal tissue. It has been proposed that JCV is transmitted mainly from parents to children through long-term cohabitation. The objective of this study was to further elucidate the mode of JCV transmission. In 5 families, we selected parent/child pairs between whom JCV was probably transmitted (judged on the basis of the identity of a 610-bp JCV DNA sequence between the parent and child). We established 5 to 9 complete JCV DNA clones from the urine of each parent or child. The complete sequences of these clones were determined and compared in each family. Nucleotide substitutions were detected in 4 parents and 1 child, and sequence rearrangements (deletions or duplications) were found in 2 parents and 2 children. Phylogenetic comparison of the detected sequences indicated that the diversity of JCV DNA sequences was generated in each family (i.e. not caused by multiple infection). We found that in 4 of the 5 families, a sequence detected in the parent was completely identical to one in the child. These findings provided further support for the proposed mode of JCV transmission, i.e. parent-to-child transmission during cohabitation.

Introduction

JC polyomavirus (JCV) is the causative agent of a fatal demyelinating disease in the central nervous system, known as progressive multifocal leukoencephalopathy (PML) [26, 29]. However, this virus is ubiquitous in the human population. Primary infection usually occurs asymptomatically during childhood [30, 40]. JCV persists in the kidney of most adults, who excrete progeny viruses in urine [1, 9, 20, 22, 39]. JCV may also persist in other sites, including peripheral blood lymphocytes, lymphoid tissues, and the central nervous system [11].

Serological studies [10, 30, 37, 40] have shown that children are infected with JCV after birth (i.e. JCV transmission is categorized as horizontal [28]). Using urinary JCV DNA, several attempts have been made to further elucidate the mode of JCV transmission. (i) Kunitake et al. [24] PCR-amplified a 610-bp JCV DNA region (IG region) from urine specimens collected from all members of 7 families. (The IG region was previously established as a region of the JCV DNA that contains relatively abundant sites for typing JCV DNAs [7].) JCV strains were identified by the nucleotide sequences of the amplified IG regions. Strains detected in half of the JCV-positive children were identified in their parents. Furthermore, Kunitake et al. [24] detected the same IG sequences in the offspring as well as in the fathers (3 cases) or mothers (3 cases) of the 6 families, suggesting that JCV transmission occurs both maternally and paternally. (ii) Kato et al. [17] studied whether JCV have been transmitted from the American population to the Japanese population both existing on a small island, Okinawa, Japan. No American JCV genotypes were detected in the Japanese population. (iii) Suzuki et al. [36] collected urine samples in Los Angeles from second and third generation Japanese-Americans whose parents and grandparents were all Japanese. From these urine samples, 2 genotypes (CY and MY) of JCV that predominantly occur in homeland Japanese [23] were mainly detected in each generation of the Japanese-Americans. Taken these findings together, it is likely that JCV is transmitted mainly from parents to children during long-term cohabitation.

In this study, we attempted to gain further insights into the transmission of JCV. We selected 5 families in each of which JCV was probably transmitted from a parent to a child (2 children in a single family). This judgment was made on the basis of the identity of the 610-bp IG sequence between the parent and child. At least 5 complete JCV DNA clones were established and sequenced from the urine of each parent or child. Although JCV DNA sequences significantly varied in most families, we detected a completely identical JCV DNA sequence in both the parent and the child belonging to each of 4 families. These findings provided support for the proposed mode of JCV transmission, i.e. parent-to-child transmission during cohabitation.

Materials and methods

Subjects

Five families were studied (Table 1). Families A to C were previously studied by Kunitake et al. [24] (families A, B, and C corresponded to families 3, 2, and 5, respectively, studied by Kunitake et al. [24]). Families D and E were first investigated in this study. A parent/child pair (2 children in family A) was selected in each family on the basis of the identity of the 610-bp IG sequence detected in their urine. All subjects were immunocompetent.

DNA analysis

Entire JCV DNAs were cloned into pUC19 at the unique *Bam*HI site as described previously [42]. The resultant complete JCV DNA clones were prepared using a QIAGEN Plasmid Midi

Table 1. Parents and children included in this study[a]

Family	Member	Age (yr)	No. of clones analyzed
A	Parent (father)[b]	52	6
A	Child 1 (daughter)	29	5
A	Child 2 (daughter)	26	5
B	Parent (mother)[c]	84	6
B	Child (daughter)	59	6
C	Parent (father)[b]	86	9
C	Child (son)	60	5
D	Parent (mother)[d]	56	5
D	Child (son)	26	5
E	Parent (mother)[d]	62	5
E.	Child (son)	25	5

[a]A parent/child pair (2 children in family A) was selected in each family on the basis of the identity of the 610-bp IG sequence detected in their urine

[b]JCV DNA was not detected in the mother

[c]No information on the JCV DNA sequence in the father was available because of his death

[d]The 610-bp IG sequence in the father significantly diverged from that in the child

kit (QIAGEN GmbH, Hilden, Germany). Purified plasmids were sequenced as described previously [35].

Phylogenetic analysis

The noncoding regulatory region of the JCV genome was excluded from phylogenetic analysis, as this region is hypervariable especially in JCV isolates derived from the brains of PML patients [16, 41, 43]. The determined and reference sequences were aligned using the CLUSTAL W program [38]. The aligned sequences were subjected to phylogenetic analysis using the neighbor-joining (NJ) method [32]. Phylogenetic trees were constructed using CLUSTAL W, and divergences were estimated by the 2-parameter method [19]. Phylogenetic trees were visualized using TREEVIEW [31]. To assess the confidence of branching patterns of the NJ trees, 1,000 bootstrap replications were performed [12]. Bootstrap probabilities larger than 70% were considered to be significant [15].

Results

Sequences detected in each family

We attempted to establish complete JCV DNA clones from urine samples collected from parents and children belonging to families A to E. We obtained 5 to 9 complete JCV DNA clones from each subject (Table 1). We sequenced all of these clones and deposited the obtained sequences in GSDB, EMBL, DDBJ, and NCBI nucleotide sequence databases with the accession numbers indicated in Table 2.

Two sequences, FA-1 and FA-1a, were detected in family A (Table 3). In reference to FA-1, FA-1a carried a 63-bp deletion within the VP2/3 gene spanning

Table 2. Complete JCV DNA sequences determined in this study

Family	Sequence	Genotype	Accession no.[a]
A	FA-1	B1-b	AB103387
A	FA-1a	B1-b	AB103402
B	FB-1	MY-b	AB103403
B	FB-2	MY-b	AB103404
B	FB-3	MY-b	AB103405
C	FC-1	CY	AB103406
C	FC-1a	CY	AB103407
C	FC-2	CY	AB103408
C	FC-3	CY	AB103409
C	FC-3a	CY	AB104487
C	FC-4	CY	AB103410
C	FC-4a	CY	AB103411
C	FC-5	CY	AB103412
D	FD-1	MY-b	AB103413
D	FD-1a	MY-b	AB103414
D	FD-2	MY-b	AB103415
D	FD-3	MY-b	AB103416
E	FE-1	CY	AB103417
E	FE-2	CY	AB103418
E	FE-3	CY	AB103419
–[b]	HA[c]	MY-b	AB103420
–	HS[c]	MY-b	AB103421
–	KF[c]	MY-b	AB103422
–	ST[c]	MY-b	AB103423

[a]GSDB, EMBL, DDBJ, and NCBI nucleotide sequence databases
[b]Unrelated to families A–E
[c]Clones previously established [40]

Table 3. Comparison and distribution of complete JCV DNA sequences detected in family A

Sequence	Rearrangement	No. of clones with the indicated sequence in:		
		Parent	Child 1	Child 2
FA-1	–[a]	6	5	4
FA-1a	del[b]	0	0	1

[a]None
[b]Deletion of a 63-bp sequence (nt 1281–1343) within the VP2/3 gene

from nucleotide (nt) 1281 to 1343 (nucleotide numbers are those of an archetype isolate, CY [18], starting from the midpoint of the origin of replication and proceeding clockwise). All clones from the parent and child 1 and most clones from child 2 contained FA-1, while a single clone from child 2 had FA-1a.

Table 4. Comparison and distribution of complete JCV DNA sequences detected in family B

Sequence	Nucleotide substitution at:		Rearrangement	No. of clones with the indicated sequence in:	
	nt 1667	nt 4109		Parent	Child
FB-1	A	A	–[a]	3	0
FB-2	G	A	–	2	6
FB-3	G	G	–	1	0

[a]None

Table 5. Comparison and distribution of complete JCV DNA sequences detected in family C

Sequence	Nucleotide substitution at:				Rearrangement	No. of clones with the indicated sequence in:	
	nt 2612	nt 3065	nt 3202	nt 3310		Parent	Child
FC-1	G	G	A	A	–[a]	0	4
FC-1a	G	G	A	A	del[b]	0	1
FC-2	G	G	A	T	–	1	0
FC-3	G	A	A	A	–	2	0
FC-3a	G	A	A	A	dup[c]	1	0
FC-4	G	G	C	A	–	3	0
FC-4a	G	G	C	A	dup[d]	1	0
FC-5	T	G	A	G	–	1	0

[a]None
[b]Deletion of a 38-bp segment (nt 4931–4968) within the LT exon
[c]Duplication of a 36-bp segment (nt 204–239) within the regulatory region
[d]Duplication of a 52-bp segment (nt 212–263) within the regulatory region

Three sequences, FB-1 to -3, were detected in family B (Table 4). These sequences were distinguishable by nucleotide substitutions at 2 sites, nt 1667 and 4109, within the VP1 and large T antigen (abbreviated as LT) genes, respectively. FB-1 to -3 were found in the parent, but only FB-2 was found in the child.

Eight sequences, FC-1, -1a, -2, -3, -3a, -4, -4a, and -5, were detected in family C (Table 5). These sequences were distinguishable by substitutions at 4 sites, nt 2612, 3065, 3202, and 3310, all locating within the LT gene, and by a 38-bp deletion within the LT gene, and 36-bp and 52-bp duplications within the regulatory region. Six sequences (FC-2, -3, -3a, -4, -4a, and -5) were found in the parent, and 2 sequences (FC-1 and FC-1a) were found in the child.

Four sequences, FD-1, -1a, -2, and -3, were detected in family D (Table 6). These sequences were distinguishable by nucleotide substitutions at 2 sites, nt 2933 and 3308, within the LT gene and by a 28-bp deletion within the regulatory

Table 6. Comparison and distribution of complete JCV DNA sequences detected in family D

Sequence	Nucleotide substitution at:		Rearrangement	No. of clones with the indicated sequence in:	
	nt 2933	nt 3308		Parent	Child
FD-1	C	A	–[a]	2	5
FD-1a	C	A	del[b]	1	0
FD-2	C	T	–	1	0
FD-3	T	A	–	1	0

[a]None

[b]Deletion of a 28-bp segment (nt 217–244) within the regulatory region. This deletion accompanied 3 nucleotide substitutions at nt 245, 247, and 253

Table 7. Comparison and distribution of complete JCV DNA sequences detected in family E

Sequence	Nucleotide substitution at:		Rearrangement	No. of clones with the indicated sequence in:	
	nt 3225	nt 3308		Parent	Child
FE-1	G	A	–[a]	2	4
FE-2	G	C	–	3	0
FE-3	A	A	–	0	1

[a]None

region. All of the four sequences were found in the parent, but only FD-1 was found in the child.

Three sequences, FE-1 to -3, were detected in family E (Table 7). These sequences were distinguishable by substitutions at 2 sites, nt 3225 and 3308, within the VP1 and LT genes, respectively. FE-1 and -2 were found in the parent, and FE-1 and -3 were found in the child.

Genotyping JCV DNA sequences detected in the 5 families

The JCV strains in the world constitute a single serotype [26]. However, they are classified into more than ten subtypes (also designated genotypes) based on nucleotide differences among them [6]. To classify detected JCV DNA sequences into genotypes, we constructed a NJ phylogenetic tree from 17 complete JCV DNA sequences detected in the 5 families together with 64 complete JCV DNA sequences reported previously [2–5, 13, 18, 25, 35]. According to the resultant tree (not shown), (i) the JCV DNA sequences detected in family A belonged to genotype B1-b; (ii) those detected in families B and D belonged to genotype

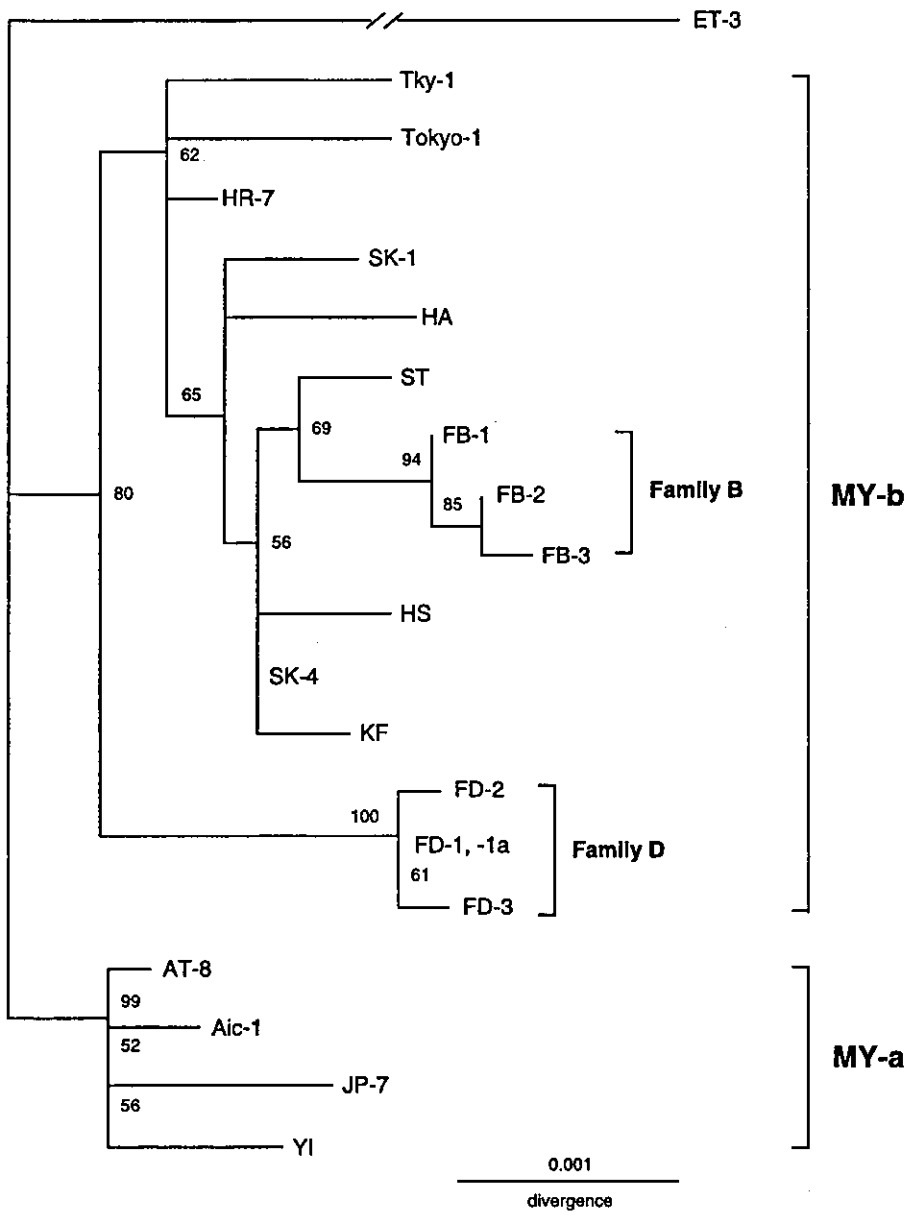

Fig. 1. NJ phylogenetic tree relating complete JCV DNA sequences detected in families B and D. A NJ phylogenetic tree was constructed from 6 complete MY-a/-b sequences detected in families B and D and 13 complete MY sequences detected in unrelated individuals (11 Japanese and 2 Koreans) [4, 18, 35, 44, and Table 2] (the noncoding regulatory region of the JCV genome was excluded from this phylogenetic analysis). The phylogenetic tree was visualized using TREEVIEW. The tree was rooted using a genotype Af2 isolate (ET-3) [35] as the outgroup. The symbols for isolates are shown in Table 2 and described elsewhere [4, 18, 35, 44]. The numbers at nodes in the tree indicate the bootstrap confidence levels (percent) obtained with 1,000 replications (only values $\geq$50% are shown)

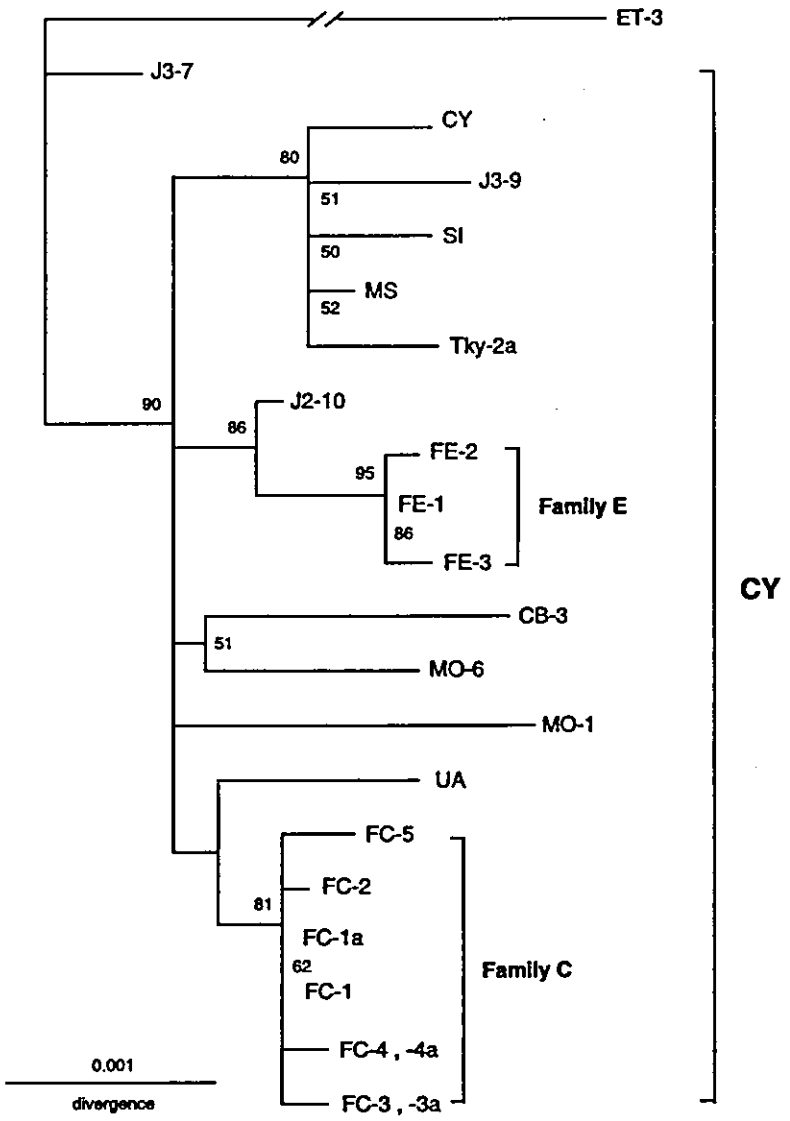

Fig. 2. NJ phylogenetic tree relating complete JCV DNA sequences detected in families C and E. A NJ phylogenetic tree was constructed from 11 complete CY sequences detected in families C and E and 11 complete CY sequences detected in unrelated individuals (6 Japanese, 3 Japanese Americans, 2 Mongolians, and 1 Chinese [18, 35, 36]. The phylogenetic tree was visualized using TREEVIEW. The tree was rooted using a genotype Af2 isolate (ET-3) [35] as the outgroup. The symbols for isolates are shown in Table 2 and described elsewhere [18, 35, 36]. The numbers at nodes in the tree indicate the bootstrap confidence levels (percent) obtained with 1,000 replications (only values $\geq$50% are shown)

MY-b; and (iii) those detected in families C and E belonged to genotype CY. B1-b is mainly prevalent in Central and Western Asia [34], but is rarely detected in Japan [23]. MY-b and CY are both prevalent in Japan [23].

Phylogenetic comparison of JCV DNA sequences in each family

As described above, all JCV DNA sequences detected in families B and D belonged to genotype MY. These sequences together with 13 complete MY-a/-b DNA sequences identified in unrelated subjects, including 11 in Japanese and 2 in South Koreans [18, 35, 44, and Table 2], were used to construct a NJ phylogenetic tree (Fig. 1). All JCV DNA sequences detected in families C and E belonged to genotype CY. These sequences together with 11 complete MY DNA sequences previously reported, including 5 in Japanese, 3 in Japanese-Americans, 2 in Mongolians, and 1 in a Chinese [18, 35, 36], were used to construct a NJ phylogenetic tree (Fig. 2). On the resultant trees (Figs. 1 and 2), all sequences detected in each family clustered together at a bootstrap probability of 75–100%. As bootstrap probabilities larger than 70% can be considered to be significant [15], the above result suggests that all JCV DNA sequences in each family were phylogenetically related.

Alteration in the amino acid sequences of viral proteins

Nucleotide substitutions detected in JCV DNA sequences and amino acid substitutions predicted by them are shown in reference to prototypical sequences

Table 8. Amino acid substitutions caused by nucleotide substitutions in complete JCV DNA clones

JCV DNA sequences	Site of the JCV genome (gene)	Nucleotide substitution[a]	Amino acid substitution[b]
FB-2, -3	nt 1667 (VP1)	A/G	–[c]
FB-3	nt 4109 (LT)	A/G	–
FC-2	nt 3310 (LT)	A/T	–
FC-3, -3a	nt 3065 (LT)	G/A	S/L
FC-4, -4a	nt 3202 (LT)	A/C	–
FC-5	nt 2612 (LT)	G/T	T/N
FC-5	nt 3310 (LT)	A/G	–
FD-2	nt 3308 (LT)	A/T	–
FD-3	nt 2933 (LT)	C/T	–
FE-2	nt 3308 (LT)	A/C	–
FE-3	nt 3225 (LT)	G/A	T/I

[a][nucleotide in the indicated JCV DNA sequence]/[nucleotide in the hypothetical prototypical JCV DNA sequence] (see text)

[b][amino acid in the LT encoded by the indicated JCV DNA sequence]/[amino acid in the LT encoded by the hypothetical prototypical JCV DNA sequence] (see text)

[c]None

in Table 8. Eight of the 11 nucleotide substitutions accompanied no amino acid change, but 3 caused amino acid substitutions.

Rearrangements (deletions) in FA-1a and FC-1a were within regions encoding viral proteins, VP2/3 and LT, respectively (Tables 3 and 5). As the deletion in FA-1a caused no frame shift, FA-1a could produce a shorter VP2/3 lacking C-terminal 21 amino acids. On the other hand, the deletion in FC-1a caused a frame shift in the first exon of LT, and therefore FC-1a would have produced a truncated LT containing only 12 N-terminal amino acids.

Discussion

In this study, we sequenced many complete JCV DNA clones in both parents and children between whom JCV was very probably transmitted. We detected multiple JCV DNA sequences in 4 of the 5 parents and in 3 of the 6 children. It is unlikely that these variations in JCV DNA sequences were introduced during molecular cloning, because of the high fidelity of DNA synthesis in bacterial cells. Phylogenetic analysis suggested that multiple JCV DNA sequences detected in each family are related to each other. As superinfection by a different JCV strain is rather rare [21], it is likely that in each family, one of the detected sequences is the prototypical one from which the others were generated by nucleotide substitution or sequence rearrangement. Although we could not definitely determine which sequence is actually prototypical in each family, it is possible that the sequences at the nodes of family-specific clusters represented the prototypical ones (Figs. 1 and 2).

On the basis of the present data shown in Tables 3, 4, 6, and 7, we could identify JCV DNA sequences that were transmitted from parents to children in families A, B, D, and E. (i) Only a single JCV DNA sequence (FA-1) was detected in the parent of family A, and this sequence was mainly found in two children. Therefore, it was inferred that a JCV strain containing FA-1 was transmitted to 2 children of family A. (ii) Three sequences (FB-1, -2, and -3) were detected in the parent, while only a single sequence (FB-2) was found in the child. Therefore, it was inferred that FB-2 was transmitted from the parent to the child. (iii) Four sequences (FD-1, -1a, -2, and -3) were detected in the parent (Table 6), while only a single sequence (FD-1) was found in the child (Table 6). Therefore, it was inferred that FD-1 was transmitted from the parent to the child. (iv) FE-1 and -2 were detected in the parent of family E, while FE-1 and -3 detected in the child. Therefore, it was inferred that FE-1 was transmitted from the parent to the child.

In family C, however, we could not identify a common sequence in the parent and the child. Six sequences (FC-2, -3, -3a, -4, 4a, and -5) were detected in the parent of family C, while 2 sequences (FC-1 and -1a) were found in the child. According to phylogenetic analysis (Fig. 2), FC-1 was found at the node of the family-C cluster. Although FC-1 was not detected in the urine sample collected from the parent in this study, we may assume that the parent carried FC-1 when he cohabited with his child (this strain may have disappeared in the parent because

of frequent changes in the genome). If this assumption can be accepted, we may infer that FC-1 was transmitted to the child of family C.

To examine the possibility that JCV is transmitted preferentially within the family, Kunitake et al. [24] PCR-amplified the 610-bp IG region from urine specimens collected from all members of 7 families. JCV strains were identified by the nucleotide sequences of the amplified IG regions. From the obtained results, the authors concluded that JCV is transmitted frequently from parents to children. Nevertheless, since JCV strains with identical IG sequences often occur in the same geographical region [8, 14, 23, 27, 33, 34], the detection does not necessarily indicate that a JCV strain was transmitted from the parent to the child. In this study, we detected a completely identical JCV DNA sequence in both the parent and the child belonging to each of the four families. We thus provided support for the proposed mode of JCV transmission, i.e. parent-to-child transmission during cohabitation.

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Stability of JC Virus Coding Sequences in a Case of Progressive Multifocal Leukoencephalopathy in Which the Viral Control Region Was Rearranged Markedly

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• *Context.*—It is generally accepted that JC virus variants in the brains of patients with progressive multifocal leukoencephalopathy are generated from archetypal strains through sequence rearrangement (deletion and duplication, or deletion alone) in the control region. This change is thought to occur during persistence of JC virus in patients.

Objective.—The present study was performed to ascertain whether amino acid substitution in the viral proteins is involved in the generation and propagation of JCV variants with rearranged control regions.

Design.—Many complete JC DNA clones were established from brain tissues (cerebellum, occipital lobe, and brainstem) autopsied in a case of progressive multifocal leukoencephalopathy in which multiple distinct control sequences were detected. Control and coding sequences were determined and compared among the JC DNA clones.

Results.—Twenty-eight control-region and 20 coding sequences of JC virus were compared. Five rearranged control sequences were detected, but they could be classified into 3 groups that shared common structural features. Viral coding sequences were identical among clones with different control regions and among clones derived from different brain regions.

Conclusion.—In the present case, nucleotide substitution in the viral coding regions (and resultant amino acid change in the viral proteins) was involved neither in the genesis of rearranged JC virus variants nor in the proliferation of demyelinated lesions in the brain.

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Progressive multifocal leukoencephalopathy (PML) is a fatal demyelinating disease of the central nervous system that affects individuals with decreased immunocompetence.[1] The causative agent is the human polyomavirus JC virus (JCV), first isolated in 1971 from the brain of a PML patient.[2] Although PML was once a rare disease, it is now a common opportunistic infection in patients with acquired immunodeficiency syndrome.[3]

Most individuals are asymptomatically infected with JCV during childhood.[4,5] The infecting JCV reaches the kidney, probably through viremia, and persists there throughout life.[6,7] In adults, the renal JCV replicates and excretes progeny in urine.[8–11] Renal JCV DNA carries the archetype control region (archetype CR),[7] whereas JCV DNA in the brain of PML patients contains various CRs (PML-type CRs) that harbor deletions and duplications, or deletions alone, in reference to the archetype.[12–15]

To explain the correlation between archetype and PML-type JCVs, Yogo and Sugimoto[15] proposed the archetype concept, which is formulated as follows: (1) JCVs with the archetype CR are circulating in the human population; (2) the archetype CR is highly conserved, in marked contrast to the hypervariable CRs (PML-type CRs) of JCVs in the brain of PML patients; (3) each PML-type CR is produced from the archetype by deletion and duplication, or by deletion alone; (4) the shift of the CR from archetype to PML type occurs during persistence in the host; and (5) PML-type JCVs never return to the human population.

The archetype concept assumes that no amino acid change in the viral proteins is involved in the generation of PML-type JCV. To examine this assumption, we studied a PML case in which multiple, distinct, PML-type JCVs were detected in autopsied brain tissues.[16] We established and sequenced many complete JCV DNA clones. Viral coding sequences were identical among clones with different control regions and among clones derived from different brain regions. We concluded that nucleotide substitution (and resultant amino acid change) was involved neither in the genesis of rearranged JCVs nor in the proliferation of demyelinated lesions in the brain.

MATERIALS AND METHODS

A detailed case report was published previously.[16] In brief, a 14-year-old boy with Wiskott-Aldrich syndrome suffered from progressive impairment of ocular movement and anarthria 6 months after allogenic bone marrow transplantation. T2-weighted magnetic resonance imaging showed high-signal areas in the right occipital lobe, cerebellum, and pons. JC viral DNA was detected in the cerebrospinal fluid by nested polymerase chain re-

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