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The Early Region of Human Papovavirus JC Induces Dysmyelination in Transgenic Mice

Judy A. Small,*† George A. Scangos,* Linda Cork,‡ Gilbert Jay,† and George Khoury†
*Department of Biology
The Johns Hopkins University
Baltimore, Maryland 21218
†Laboratory of Molecular Virology
National Institutes of Health
Bethesda, Maryland 20892
‡Division of Comparative Medicine
Department of Pathology
The Johns Hopkins Medical Institute
Baltimore, Maryland 21205

Summary

Transgenic mice containing the early region of human papovavirus JC were produced. Some of these mice exhibited a shaking disorder similar to the previously described mutant mice *jimpy* or *quaking*. Neuropathological analysis indicated a dysmyelination in the central nervous system, but not the peripheral nervous system. A high level of JCV T-antigen mRNA was present in the brains of the mice exhibiting the myelin disorder. JC virus is associated in humans with a degenerative demyelinating disease: progressive multifocal leukoencephalopathy. The JCV-containing transgenic mice may therefore provide an animal model for studying this disease.

Introduction

JC virus is a papovavirus which is widespread in human populations (Gardner, 1973; Padgett and Walker, 1973). It has been implicated as the causative agent in the chronic demyelinating disease progressive multifocal leukoencephalopathy (PML) (Johnson, 1983; Walker, 1978; ZuRhein, 1969). PML is an opportunistic disease that generally affects patients already exhibiting an immunocompromised condition due to illness, genetic disorders, or therapeutic immunosuppression for transplants (Johnson, 1983; Walker, 1978). JC viral particles have been isolated from brain lesions of patients with PML, in which there are abnormal oligodendrocytes and astrocytes (Astrom et al., 1958; Johnson, 1983; ZuRhein, 1969). Demyelination is caused by destruction of oligodendrocytes, the myelin-producing cells of the brain. JC virus (JCV) has also been postulated to be involved in the formation of multiple glial tumors in some patients with PML (Castaigne et al., 1974; GiaRusso and Koeppen, 1978; Richardson, 1961). When inoculated into newborn hamsters, JCV causes a wide variety of tumors (Padgett et al., 1977; Varakis et al., 1978; Walker et al., 1973), most of which are derived from neural tissue. It is the only human virus that has been demonstrated to cause tumors in primates, namely owl monkeys (London et al., 1978).

Transgenic mice containing JCV early region genes under the control of JCV promoter/enhancer elements were generated. Ten transgenic mice containing intact JCV early region sequences were obtained, although five of the ten were born dead. Of the remaining five, all three females succumbed to tumors at 14–16 weeks of age, and one of two males died with tumors at ten months of age (Small et al., unpublished data).

The study described in this paper characterized the offspring derived from two male JCV-containing founder mice. One mouse, JC48, lived for ten months before dying as a result of tumorigenesis. The other, JC91, lived for four and a half months and died of causes unrelated to tumor formation. Both of these mice produced offspring that manifested an unusual phenotype. JC48 offspring were apparently normal at birth, but at two weeks of age developed a severe tremor that was evident while the mice were moving, but not when they were at rest. The tremor became progressively more severe as the mice aged. At approximately three weeks of age, the mice exhibited tonic seizures, and died at four to six weeks. JC91 offspring developed a similar, but milder, form of this tremor at three to four weeks of age; these mice lived for two to five months. This phenotype resembles that of the myelin-deficient strains of *quaking* and *jimpy* mice (Hogan, 1977; Sidman et al., 1964). Neuropathological examinations of the affected mice indicated a myelin deficiency in the central nervous system that appears to correlate with the expression of JCV T-antigen in brain tissue.

Results

In these experiments, the JCV early region (see Figure 1A), encoding large and small T-antigens and containing the JCV enhancer/promoter region (including two 98 bp repeats), was injected into one-cell mouse embryos. Five founder mice containing intact JCV early region sequences were obtained (Table 1). Three female founder mice succumbed to tumors, diagnosed as neuroblastomas of the adrenal medulla, at 14–16 weeks of age, and one male founder developed similar tumors at ten months of age (Small et al., unpublished data). All three females produced offspring, but the low transmission frequencies of JCV DNA sequences to offspring indicated that all were germ-line mosaics. Of the two males, the one that developed the tumors (founder JC48) was also a germ-line mosaic while JC91 was not. No lines of mice were generated from the three female transgenic mice containing intact copies of the JCV early region.

Two lines of transgenic mice (JC48 and JC91) that contained the JCV early region genes were established. In both lines intact copies of JCV control region and T-antigen gene were demonstrated by the appearance of 2.54 and 0.33 kb bands after PvuII digestion (Figure 1) and by analysis with several other restriction enzymes (data not shown). Both founder mice produced numerous offspring. There were two separate JCV integration sites in the genome of founder JC48, as determined by the patterns of

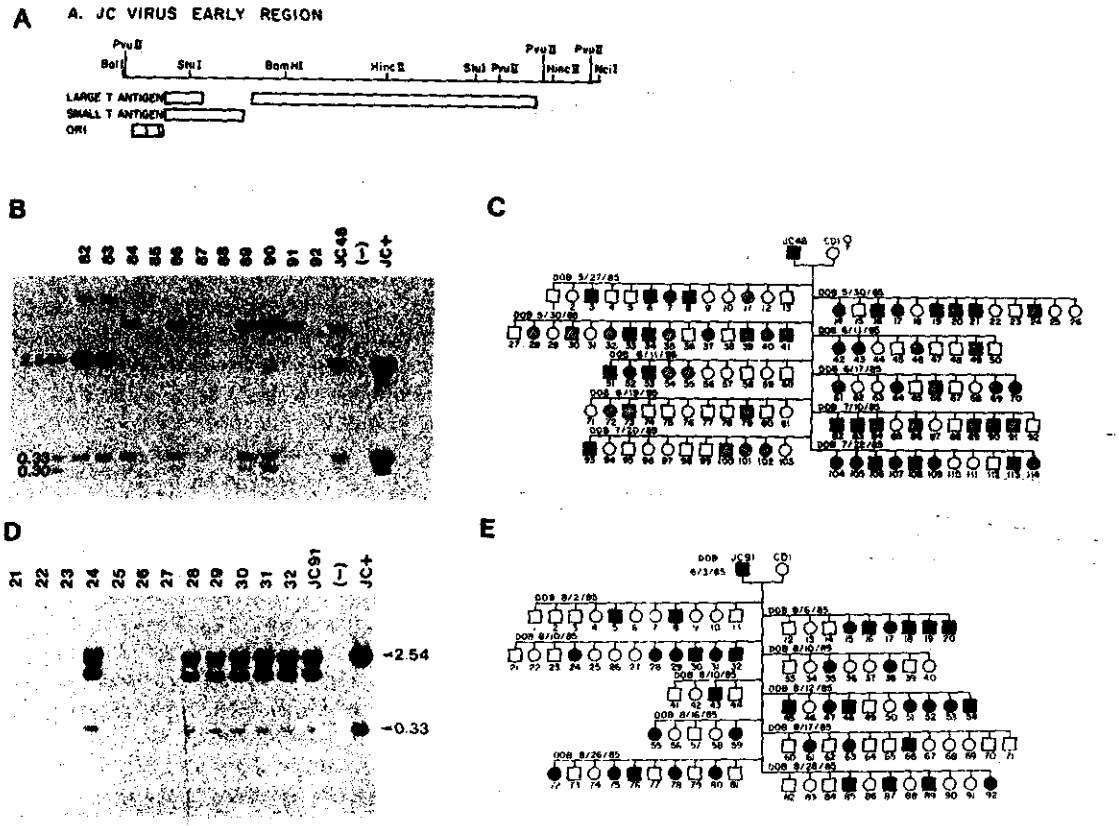


Figure 1. Inheritance of JC Virus Sequences in JC48 and JC91 Offspring

(A) Maps of the JC virus early region. The BalI/NciI fragment contains the enhancer/promoter elements, labeled ORI, and T-antigen coding regions of the virus as indicated. Restriction sites are indicated above the line.

(B) and (D) Southern analysis of representative litters from JC48 and JC91. Tail DNA was digested with PvuII, electrophoresed through a 1% agarose gel, transferred to nitrocellulose, and hybridized with a ^{32}P-labeled JCV early region probe.

(B) Offspring from JC48. JC48 is the founder male, (−) is a negative control mouse, and JC+ is (−) with 50 pg of added JCV early region DNA. Mice 82 and 83 contain 2.54 and 0.33 kb fragments corresponding to the intact T-antigen gene. Other positive mice do not contain intact JCV early region genes.

(D) Offspring from JC91. JC91 is the founder male, and (−) and JC+ are as in (B). Mice numbered 24 and 28–32 all contain intact JCV early region genes.

(C) and (E) Pedigrees showing inheritance of JCV sequences in the first ten litters of JC48 and JC91. Each litter was produced by crossing the male founder with a different CD1 female mouse. Closed symbols indicate mice that were positive for intact JCV early region genes, cross-hatched symbols are mice that inherited partial sequences, and open symbols are negative mice.

(C) Offspring of JC48.

(E) Offspring of JC91.

transmission (Figure 1B). Of the offspring, 30% received copies of the integration site which did not contain an intact JCV early region, while 20% received copies of the second site which did contain intact sequences (Figure 1C, Table 1). No mouse inherited both integration sites present in the founder JC48, suggesting that the founder male was a germ-line mosaic. The JCV DNA copy number in the mice inheriting the intact sequences was five to ten per cell, apparently arranged as tandem copies at one locus (our unpublished data).

JC91 transmitted JCV DNA sequences to approximately 50% of its offspring (Figure 1E, Table 1). The hybridization patterns of this founder male and his positive offspring are indistinguishable (Figure 1D); about ten to 20 copies of the JCV sequences are present per cell, again apparently arranged in tandem at a single locus (data not shown).

Table 1. Characteristics of JCV Mice

Founder Mouse	Transmission to Offspring	Tumors in Founders	Neurological Disorder	
			Founders	Offspring
JC48♂	19% (22/114)[a]	+	−	+
JC62♀	16% (3/18)[b]	+	−	ND[c]
JC74♀	33% (1/3)[d]	+	−	ND
JC86♀	4.5% (1/22)	+	−	+
JC91♂	41% (38/92)	−	+	+

[a] Two integration sites were transmitted independently to the offspring, with only 19% receiving intact copies of JCV sequences, while 31% received partial copies (see Figure 1C).

[b] Three out of 18 were positive for JCV sequences; however all three were aborted when the founder mouse died.

[c] ND: not determined.

[d] One of three was positive, but was born dead.

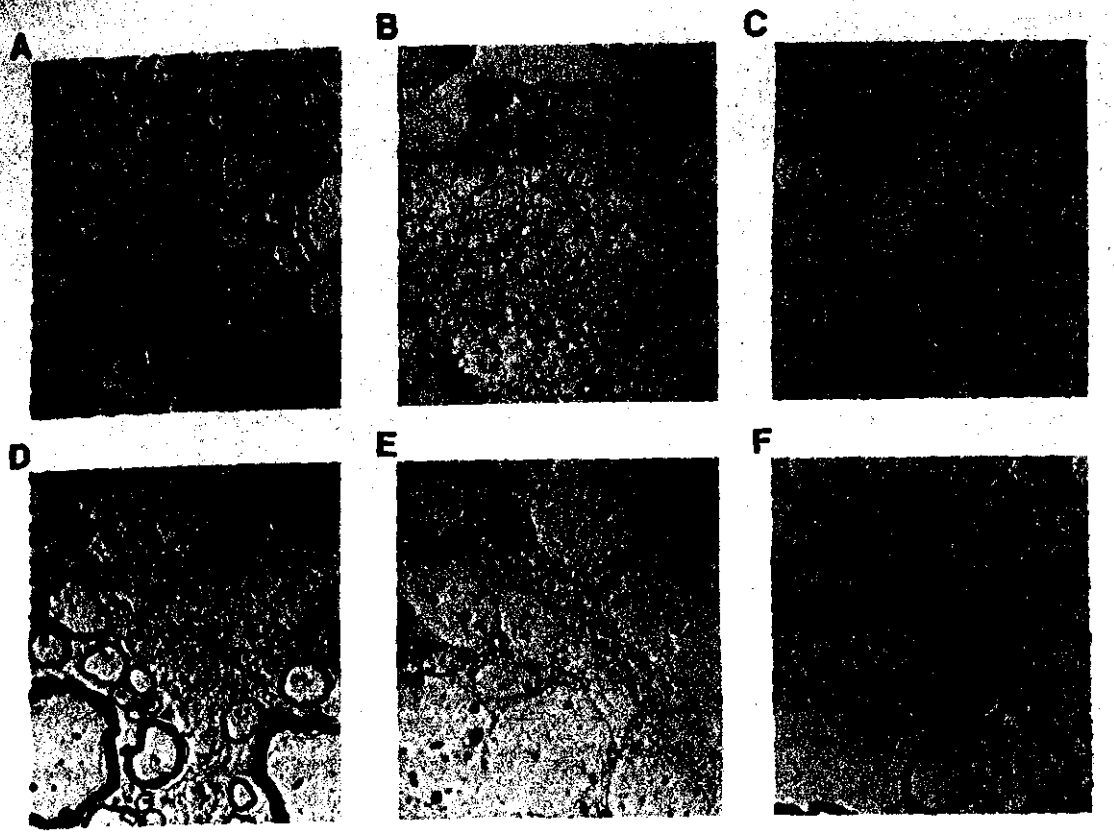


Figure 2. Neuropathological Analysis of the Spinal Cord of JC48 and JC91 Offspring

Tissue was obtained from four week old mice, fixed in paraformaldehyde, embedded in epon, sectioned, and stained with uranyl acetate.

(A) and (D) Cross-sections of several axons of the spinal cord of a negative control mouse. The electron dense material surrounding the axons is the myelin sheath.

(B) and (E) Cross-sections of the spinal cord from a JC48 offspring. There is a definite lack of myelin around most axons. A lipid-containing macrophage is indicated by the large arrowhead in (B).

(C) and (F) Cross-sections of a spinal cord from a JC91 mouse. There is myelin around most axons, but note the numerous vacuoles and noncompacted myelin. A bizarre oligodendrocyte nucleus is present in (F) (indicated by an arrow). (A), (B), and (C): 3000X. (D), (E), and (F): 6000X.

The offspring of JC48 appeared normal at birth. At two weeks of age, a small number developed a characteristic tremor. They were not affected while at rest, but shook vigorously when moving. Upon analysis of tail DNA, only the shaking mice contained intact copies of the integrated JCV T-antigen coding sequences. At about three weeks of age, these mice began to exhibit tonic seizures which lasted for 15–30 sec. These characteristics are similar to those of several myelin-deficient strains of mice, including *jimpy* and *quaking* (Hogan, 1977; Sidman et al., 1964). Most affected mice died by four weeks of age, and none lived for more than six weeks. Since none of the affected mice reached sexual maturity, a permanent line of JC48 mice could not be generated.

Founder mouse JC91 and its offspring exhibited a similar, but less severe form of the shaking disease. A slight tremor became noticeable three to four weeks after birth. In general, the mice from this line were healthier than mice from JC48. Affected offspring lived for two to five months and a number have been successfully bred. JCV DNA-containing offspring can be routinely generated from the F1 offspring, and a permanent line has been established for JC91. Homozygous mice have also been obtained from the JC91 line, and these mice exhibit a more severe shaking phenotype than their heterozygous littermates.

A third mouse, JC86 (one of the female mice that succumbed to tumors) produced a number of offspring, one of which contained intact JCV T-antigen coding sequences. This offspring also exhibited the shaking phenotype, but died before a more extensive analysis could be performed.

To determine the cause of the phenotypic abnormality, we performed a histological analysis. Neuropathologic examination of the brain, spinal cord, and peripheral nerve roots of a mouse from the JC48 line at three weeks of age showed significant hypomyelination in the central nervous system; the peripheral nerve myelin, however, appeared normal. By electron microscopy, most axons within the spinal cord did not have myelin sheaths of normal thickness or compactness (Figures 2B and 2E), compared with those of a normal littermate (Figures 2A and 2D). Large

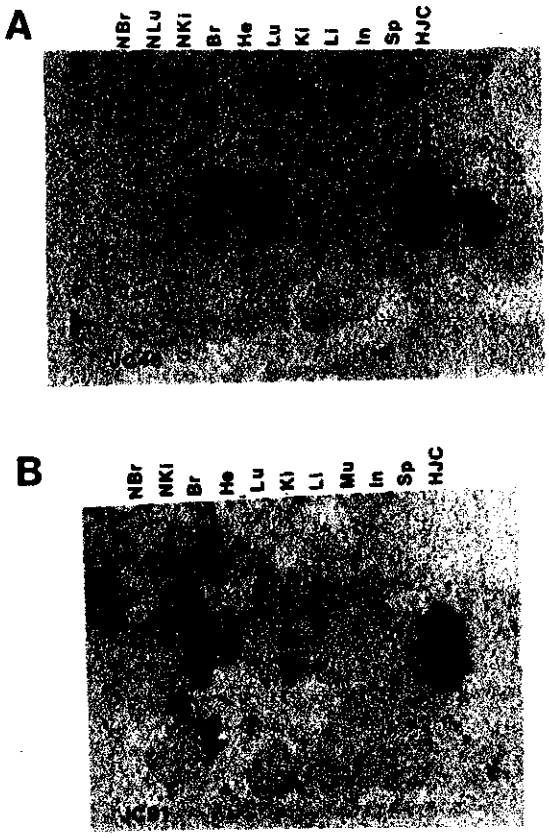


Figure 3. Northern Blot Analysis of RNA from Tissues of JC48 and JC91

Total RNA was isolated and polyadenylated RNA was selected by oligo(dT)-cellulose chromatography. Three micrograms of poly(A)$^+$ RNA was electrophoresed in a formaldehyde/agarose gel, transferred to nitrocellulose, and hybridized to a JCV probe. BR: brain. HE: heart. LU: Lung. KI: kidney. LI: liver. MU: muscle. IN: intestine. SP: spleen from a JCV-containing mouse. NBR, NLU, and NKI, are the same tissues from a negative mouse, and HJC is RNA from a JCV-transformed hamster cell line.

(A) JC48 offspring. (B) JC91 offspring.

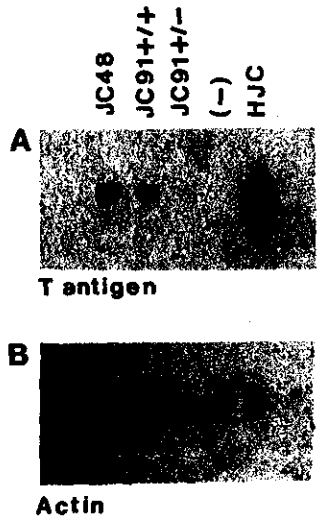


Figure 4. Comparison of the Level of JCV T-Antigen RNA Expression between JC48 and JC91 Mouse Lines

Brain mRNA was prepared as described in Figure 3.

(A) Filter hybridized with a JCV early region probe.

(B) The same filter stripped and rehybridized with a human β-actin probe. The level of total mRNA was consistent between the different lanes. Lane 1: JC48. Lane 2: JC91 homozygote. Lane 3: JC91 heterozygote. Lane 4: negative littermate. Lane 5: HJC cells.

axons had no myelin lamellae or, at the most, one or two noncompacted lamellae; oligodendrocytes often had large, bizarre nuclei. Phagocytosis of myelin by macrophages was a prominent feature (Figure 2B). These observations indicate that the myelin produced by the oligodendrocytes was abnormal. Examination of tissues of a mouse from the JC91 line also demonstrated that the central nervous system myelin was clearly abnormal, while the peripheral myelin appeared normal (Figures 2C and 2F). The white matter was studded with numerous vacuoles which, according to electron microscopy, were within the myelin lamellae surrounding the axons. Hypomyelination was detected as segmental demyelination of axons in longitudinal sections (data not shown). As was seen in JC48 mice, there were abnormal oligodendrocytes and phagocytosis of myelin. All of these changes suggested a similar but less severe phenotype for the JC91 offspring when compared to the JC48 offspring.

Expression of JCV T-antigen was assayed by RNA analysis in three week old offspring from JC48 or JC91 (Figure 3). In the JC48 mouse, JCV T-antigen encoding RNA was detected at high levels in brain and lung, and at lower, but detectable, levels in heart and kidney; there was no detectable T-antigen-specific RNA in liver, intestine, or spleen (Figure 3A). In the JC91 mouse, a high level of RNA expression was found in brain, and lower levels in heart and kidney (Figure 3B). There was no detectable JCV RNA expression in lung, liver, muscle, intestine, or spleen. Hybridization to the same filters with an actin probe indicated that comparable amounts of RNA were present in each lane (data not shown). Comparison of JCV T-antigen expression in brain tissue between JC48 offspring and JC91 mice that were heterozygous or homozygous for JCV sequences indicated that JC48 mice expressed higher levels of T-antigen than JC91 mice, and that homozygous JC91 mice expressed higher levels than heterozygous littermates (Figure 4). This observation suggests that the level of T-antigen may be responsible for the differential severity of neurological symptoms in the animals.

Discussion

Although human papovavirus JC is associated with a progressive demyelinating disease and neurologic tumors in humans (Johnson, 1983; Walker, 1978; ZuRhein, 1969), JC viral particles have not been shown to induce any detectable pathology when inoculated into mice (Tooze, 1981). Transgenic mice have provided a means with which

to bypass host range restrictions and obtain JCV sequences in every cell of the mouse. Five mice containing the JCV early region sequences were generated in our laboratory. All five expressed JCV sequences, and displayed at least one of the two phenotypes. Four mice developed tumors, all neuroblastomas of the adrenal medulla (our unpublished data). The three females died at 14–16 weeks of age, and one male died at ten months of age because of metastasis of the tumors. Three mice produced offspring with a neurological abnormality; the mice appeared normal until two to four weeks of age when a characteristic shaking phenotype developed.

Two mutant strains of mice, *quaking* and *jimpy*, exhibit a similar phenotype (Hogan, 1977; Sidman et al., 1964). Both develop a tremor which is more severe upon movement, and both eventually manifest tonic seizures. Of the two mutant strains, the disease associated with *jimpy* is more severe than *quaking*: The *jimpy* mutation is a homozygous lethal, and the affected mice die at two to four weeks of age. The homozygous *quaking* mice are healthier, and females are able to produce litters. *Jimpy* and *quaking* mice, therefore, seem to resemble the two JCV lines (JC48 and JC91, respectively). The JC48 mice are more severely affected than the JC91 mice; only the latter survive to reproductive age.

In this study, the correlation between a shaking phenotype and the inheritance of intact copies of the JCV sequences is absolute. All shaking mice inherited an intact early region and all normal mice did not. These two mouse lines, JC48 and JC91, and one other independently derived mouse exhibit similar phenotypes and yet have different integration sites for the JCV sequences. The data therefore suggest that expression of JCV sequences, rather than insertional mutagenesis, is responsible for the abnormal phenotype. Although T-antigen expression was detected in several tissues of the JCV-containing m ce, there was a uniformly high level of RNA expression in the brain only. Also, the level of expression of the T-antigen sequences correlated with the severity of the shaking phenotype. Further support for this idea derives from the observation that homozygous JC91 mice not only have a more severe disease than heterozygous mice from this line, but also express the T-antigen transcript at a higher level.

Neuropathological examination of the mice indicated that the myelin was abnormal in the central nervous system, but not in the peripheral nervous system. The amount of myelin was reduced, and there were vacuoles in the myelin sheaths. This may not represent active demyelination, i.e., destruction of myelin sheaths, but rather a dysmyelination in which formation of the sheath is defective. The *quaking* and *jimpy* strains of mice also have myelin deficiencies restricted to the central nervous system (Hogan, 1977; Meier and Bischoff, 1974; Meier et al., 1974; Sidman et al., 1964; Torii et al., 1971; Wisniewski and Morrell, 1971) which are attributed to dysmyelination. Neuropathological analysis of these mice indicated structural defects in the myelin similar to those observed in the JCV-containing mice (Meier and Bischoff, 1974; Meier et al., 1974, Torii, 1971; Wisniewski and Morrell, 1971). There are large axons with no myelin, and abnormal nuclei in oligodendrocytes. Longitudinal sections show that hypomyelination is segmental. There are also defect the myelin sheaths, similar to the vacuoles observe JC91 mice as a result of improper compacting of mye At the present time, however, it seems likely that these r tant phenotypes harbor one or more defects in spec myelin genes.

The disease progressive multifocal leukoencephalo thy (PML) is characterized by a degenerative demyelir tion in the central nervous system (Johnson, 1983; Walk 1978; ZuRhein, 1969). Both oligodendrocytes and asti cytes are abnormal, and focal lesions with demyelinati are present (Astrom et al., 1958; Johnson, 1983; ZuRhei 1969). JCV has been routinely isolated from these focal l sions, and is expressed in the cells around the lesior (Padgett et al., 1971). Our study suggests that demyelin tion may be a direct result of the expression of JC T-antigens in the brain, perhaps in the oligodendrocyte The cell specificity of JCV expression would appear to b a function of the JCV enhancer element (Kenney et al. 1984). JCV early gene products may specifically impai the ability of the oligodendrocytes to synthesize myeli proteins, reduce their ability to produce proper myeli sheaths, or interfere in a more general way with cellula functions. Whether this is related to transforming proper ties of papovavirus T-antigens is unclear. Preliminary ex periments indicate that there is a decreased level of my elin proteins in the shaking mice derived from JC48 and JC91 (our unpublished results). The level of proteolipid protein (PLP) is reduced, as is that of myelin basic protein (MBP). Myelin-associated glycoprotein (MAG) is present in the cells, but may be improperly processed, such that it is not correctly inserted into the cell membrane.

While introduction of wild-type SV40 into transgenic mice can induce the development of choroid plexus papillomas (Brinster et al., 1984; Small et al., 1985), it has been shown that some transgenic mice containing the SV40 T-antigen regulated by a mouse metallothionein promoter develop a peripheral demyelinating neuropathy (Messing et al., 1985; Palmiter et al., 1985). We suspect that dysmyelination in JC48 and JC91 mice results from the general toxicity or the potential for dedifferentiation caused by a papovavirus T-antigen. Restriction of this disease to the central nervous system, on the other hand, would appear to be dictated by the host range specificity of the JCV enhancer/promoter element (Kenney et al., 1984) which restricts transcriptional activity to a subset of neural cells. This suspicion is based in part on our observations that JCV can progress through a lytic cycle in nonneural primate cells if provided with SV40 T-antigen (our unpublished results). Thus the host range restriction of JCV to human fetal glial cells would appear to be controlled at the level of early transcription that depends on the JCV enhancer element. Nevertheless, experiments in progress with chimeric constructs, such as the JCV enhancer/promoter element plus the coding sequences for SV40 T-antigen, should help to delineate the relative role of the viral control sequences and the viral T-antigens in these myelin abnormalities.

Experimental Procedures

Production of Transgenic Mice

Transgenic mice were produced as described by Gordon and Ruddle (1983). Approximately 100–1000 copies of the JCV early region, included in a 3.2 kb BalI/NciI restriction fragment, were injected into fertilized ova at the single-cell stage. Mice were screened for the acquisition of JCV DNA sequences by analysis of DNA obtained from tail biopsies.

Nucleic Acid Analysis

DNA was isolated from tail biopsies by a proteinase K/SDS extraction procedure that has been described previously (Small et al., 1985). Purified DNA (10 μg) was digested with PvuII, electrophoresed in a 1% agarose gel, transferred to nitrocellulose filters, and hybridized with a ^{32}P-labeled probe of the JCV early region. RNA was extracted by guanidinium isothiocyanate/CsCl gradient technique (Chirgwin et al., 1979). Polyadenylated mRNA was prepared by oligo-(dT)-column chromatography, and 3 μg were subjected to electrophoresis in a 0.9% agarose/formaldehyde gel. After transfer of RNA to nitrocellulose, the filters were hybridized with a JCV early region probe. Filters were washed in boiling water and rehybridized with a human β-actin probe to control for the amount of RNA in each lane.

Histological Analysis

Tissues were fixed by perfusing the animals with paraformaldehyde, and were embedded in epon. After sectioning, the tissues were stained with uranyl acetate, and examined by electron microscopy.

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