

Detection of JC Virus in Cerebrospinal Fluid (CSF) Samples from Patients with Progressive Multifocal Leukoencephalopathy but Not in CSF Samples from Patients with Herpes Simplex Encephalitis, Enteroviral Meningitis, or Multiple Sclerosis

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Received 15 October 1997/Returned for modification 17 December 1997/Accepted 5 January 1998

JC virus (JCV) DNA was detected in cerebrospinal fluid (CSF) samples from patients with progressive multifocal leukoencephalopathy (PML) but not in CSF samples from patients with herpes simplex encephalitis, enteroviral meningitis, or multiple sclerosis. This suggests that inflammatory processes in the brain do not necessarily reactivate JCV, which further supports the proposal that the presence of JCV DNA in the CSF is diagnostic for PML.

Progressive multifocal leukoencephalopathy (PML) is a rare disease observed in immunosuppressed patients previously infected with JC virus (JCV) (15). In PML, JCV lytically infects myelin-producing oligodendrocytes. This leads to severe neurological symptoms and mental deterioration in the affected patients, and the disease has a lethal outcome (11). The precise mechanism of JCV reactivation in PML is not known, but one possibility is that JCV persisting in the brain after primary infection may be reactivated (4, 13, 19). A definite diagnosis of PML requires detection of JCV in brain tissue obtained by brain biopsy, and this is not readily performed. Recently, it has been shown that it is possible to detect JCV in the cerebrospinal fluid (CSF) from patients with PML (confirmed by brain biopsy) by using a JCV-specific PCR (5, 9, 12, 18). Thus, JCV replicating in the brain is also detected in the CSF, and the presence of JCV in CSF has been proposed to be diagnostic for PML (5, 9, 12, 18).

Nevertheless, the presence of JCV in CSF samples from patients with other pathological conditions in the central nervous system (CNS) has not been examined extensively. It is not known if a latent virus like JCV possibly present in the brain could be activated during an inflammatory process in the CNS caused by other viruses. Furthermore, the possibility that JCV, which can cause demyelination in the brain, may also replicate in the brains of multiple sclerosis (MS) patients has not yet been completely excluded. To determine if JCV is activated in the brain during other viral infections in the CNS, or in MS, we examined CSF samples from patients with herpes simplex virus type 1 (HSV-1) encephalitis, enteroviral and nonenteroviral meningitis, and MS for the presence of JCV DNA.

Forty-three CSF samples from 39 patients with HSV-1 encephalitis, 20 CSF samples from 20 patients with enteroviral meningitis, and 15 CSF samples from 15 subjects with nonen-

teroviral meningitis were assayed for the presence of JCV DNA. In addition, 58 centrifuged CSF samples (45 samples collected during an exacerbation and 13 samples collected during remission) from 45 patients with early MS were also examined for the presence of JCV DNA. As positive controls, CSF samples previously described as JCV DNA positive from four AIDS patients with clinical and histopathological diagnoses of PML were used (9).

The diagnosis of HSV-1 encephalitis in all 39 patients examined was confirmed by an HSV-1 DNA-specific nested PCR (1). HSV-1 DNA was present in the CSF of all 39 patients, as indicated in Table 1. CSF samples from 35 patients (ages, 1 month to 67 years) with aseptic meningitis were examined by virus isolation for the presence of enterovirus infection (6), and 29 of these CSF samples were also examined by an enteroviral RNA-specific PCR (3, 7). Eighteen of the 35 CSF samples were positive by virus isolation, and of the 18, 15 were tested and found positive with an enterovirus-specific RNA PCR (3, 7). The isolated enterovirus types, determined by a complement fixation test for 18 patients (8), were ECHO 9 (2 patients), ECHO 11 (2 patients), ECHO 25 (1 patient), ECHO 30 (7 patients), Coxsackie B3 (1 patient), Coxsackie B4 (1 patient), and Coxsackie B5 (4 patients). Of the remaining 17 patients, whose CSF samples were negative by enterovirus isolation, 14 were tested with an enteroviral PCR. Two of these were enterovirus RNA positive, and 12 remained negative. In summary, 20 of 35 patients with aseptic meningitis had confirmed enteroviral infections by culture (6) and/or PCR (7).

The 45 MS patients (ages, 21 to 55 years) had laboratory-supported diagnoses of definite MS or clinically definite MS (16) and were rated according to an expanded disability status scale (10). The patients were ambulatory and had expanded disability status scale scores of <4 (10), and they had suffered from MS between 5 days and 5 years (median, 4 months). CSF samples were collected within 4 weeks after an exacerbation, defined as a sudden appearance of MS-related symptoms and signs or worsening of previous findings lasting more than 24 h. Routine CSF examinations revealed mononuclear pleocytosis (>5 × 10⁶ cells/liter) in 21 patients (47%) and oligoclonal

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TABLE 1. Absence of JCV DNA in CSF samples from patients with HSV-1 encephalitis

Time after onset of symptoms (days)	CSF samples ^a		
	No.	% HSV-1 PCR positive	% JCV PCR positive
1-3	8	100	0
4-7	14	100	0
>8	5	100	0
ND ^b	16	100	0
Total	43	100	0

^a CSF samples from 39 patients from 7 months to 86 years old were examined.

^b ND, not defined.

immunoglobulin G bands in only the CSF in all patients (14). None of the patients had been treated with immunosuppressive or antiviral drugs, including corticosteroids and beta interferon.

All CSF specimens were stored at -20 or -70°C before testing. Each CSF sample was divided into 10- μl duplicates and heated at 95°C for 10 min before undergoing PCR. As a positive control, a CSF sample containing JCV DNA from a patient with PML from a previously described study was used (9), as well as unheated urine samples (1 to 5 μl) from organ transplant patients which contained JCV DNA or BK virus (BKV) DNA. A nested PCR specific for the early regions of both JCV and BKV was used in this study (2). Since JCV has 75% genome homology with BKV, another human polyomavirus, the JCV (173-bp) and BKV (176-bp) amplification products were distinguished by *Bam*HI restriction enzyme cleavage. Only the JCV DNA amplicon was cleaved into two fragments (120 and 53 bp long) (2). The sensitivity of the PCR assay was 10 genome copies for both of the viruses.

All 43 CSF specimens (sampled between day 1 and more than 1 week after the onset of symptoms) from the 39 patients with HSV-1 encephalitis confirmed by an HSV-1-specific PCR technique (1) were negative for JCV DNA (Table 1). Furthermore, no JCV DNA could be detected in the CSF specimens from the 20 patients with confirmed enteroviral meningitis or from the 15 patients with meningitis of unknown etiology. In addition, no JCV DNA could be detected by PCR in the CSF specimens of the 45 patients with definite early MS. As expected, JCV DNA was present in the CSF samples of the four patients with PML (data not shown).

In summary, as in our previously published study (9), we could detect JCV DNA in the CSF samples from patients with PML. However, JCV DNA was not detected in CSF samples from patients with HSV-1 encephalitis, enteroviral or nonenteroviral meningitis, or MS. Our findings thus further support the proposal that detection of JCV DNA in the CSF is diagnostic for PML. Since the seroprevalence of JCV is around 75% (17), we had assumed it would be possible to detect JCV DNA in the CSF if JCV was indeed reactivated in the brains of patients with HSV-1 encephalitis, enteroviral meningitis, aseptic meningitis of unknown etiology, or MS. However, this was not the case. Our results therefore indicate that inflammation in the brain observed during the conditions listed above does

not necessarily cause reactivation of JCV. Thus, in order to achieve an activation of JCV in the brain and to cause PML, immunosuppression alone or in combination with other unknown factors rather than simply an inflammation in the CNS is necessary.

This work was supported by the Karolinska Institute.

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