VIROLOGY

JC virus detection in the cerebrospinal fluid of AIDS patients with progressive multifocal leucoencephalopathy and monitoring of the antiviral treatment by a PCR method

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Twenty-four cerebrospinal fluid (CSF) samples from 19 AIDS patients with neurological signs were analysed by the polymerase chain reaction (PCR) for the presence of JC virus (JCV). Eleven of the 19 patients tested presented with progressive multifocal leucoencephalopathy (PML). Two specific JCV target sequences were used for the PCR analysis: a sequence specific for the T antigen genes from both BK virus (BKV) and JCV (PCR1) and a sequence specific for the large T antigen gene from JCV (PCR2). The JCV genome was detected in 10 of 11 patients with PML by the PCR1 method and in all 11 patients by the PCR2 method. With samples from the eight patients without PML, one positive result was obtained with the PCR1 method and this sample and another gave positive results with PCR2. Multiple CSF samples were collected from three patients with PML at different times, including after intrathecal cytarabine treatment, and were tested by the PCR2 method for the presence of the JCV genome. The PCR result became negative for two of the three patients during the cytarabine treatment. However, the absence of a PCR signal was not associated with clinical improvement in these patients. The PCR method is useful for the detection of JCV in CSF samples and in the diagnosis of PML. However, the application of PCR for monitoring the effect of treatment remains to be established.

Introduction

The JC virus (JCV), a polyomavirus, is aetiologically linked with progressive multifocal leucoencephalopathy (PML), a severe subacute demyelinating disease of the central nervous system (CNS). PML is a rare complication of immunosuppressive treatment, but the general epidemiology of PML has run parallel to that of AIDS. The incidence of PML in patients with AIDS is estimated to range from 4 to 7% [1]. The classic triad of the disorder (dementia, hemiparesis, hemianopia) develops insidiously and death usually follows within 9 months. Brain biopsy remains the definitive diagnostic procedure. Besides characteristic histological features in such specimens, the viral antigen can be detected by immunofluorescence and the viral genome by in-situ hybridisation. Viral culture is often unsuc-

cessful. Among the non-invasive procedures, magnetic resonance imaging and computed tomography are useful for detecting the demyelinating lesions of PML [2]. Amplification of the viral genome by the polymerase chain reaction (PCR) has the advantage of detecting low concentrations of the viral genome. However, PCR studies have demonstrated the presence of JCV in brains of AIDS patients without clinically evident PML [3]. The aim of this study was to evaluate the diagnostic significance of a qualitative PCR method for the detection of JCV in the cerebrospinal fluid (CSF) of AIDS patients. CSF samples from patients with PML were also collected at different periods of time after intrathecal cytarabine treatment and tested by the PCR method for the presence of the JCV genome.

Patients and methods

Patients and samples

Twenty-four CSF samples from 19 AIDS patients with neurological symptoms were analysed. Eleven of the

patients were considered to have PML on the basis of clinical symptoms, neuro-imaging and absence of any other infectious process. Neurological symptoms suggestive of PML were progressive motor weakness, ataxia, visual disturbance and dementia, and magnetic resonance imaging (MRI) features suggestive of PML were altered signals from subcortical lesions. The differential diagnosis between PML and HIV encephalitis was mainly established by MRI. The localisation of lesions in the subcortical white matter and their hypointensity in the T1-weighted images favoured the diagnosis of PML *versus* HIV encephalitis in these patients. Brain biopsy or necropsy were performed in seven of these 11 patients and the histopathological features were consistent with PML. CSF samples from three PML-positive patients were collected at different time periods, including after intrathecal cytarabine treatment.

CSF samples from eight AIDS patients with other neurological conditions were tested as PML-negative controls. Additional positive and negative JCV controls were used in every PCR reaction. CSF from a patient with necropsy features consistent with the presence of PML, virions visualised by electron microscopy and positive in-situ hybridisation (patient 'Nog', Table 1) was used as a positive control. CSF from an immunocompetent patient with pneumococcal meningitis was used as a negative control. The CSF samples were stored at -20°C until tested.

DNA extraction

DNA was extracted from CSF by two methods. (1) CSF (200 μl) was boiled for 10 min and frozen for another 10 min. The boiling-freezing process was repeated three times. (2) CSF (200 μl) was mixed with 400 μl of a DNA extraction reagent (Perkin Elmer, NJ, USA)

consisting of Chelex 100 (an anion exchange resin) 20% w/v, suspended in 10 mM Tris-HCl, pH 8.0, 1.0 mM EDTA, sodium azide 0.1%. The mixture was boiled for 20 min and centrifuged at 10 000 g for 20 min at 4°C . The supernate was precipitated with ethanol (2.5 volumes) in the presence of 5 M NaCl (0.04 volumes). After centrifugation at 10 000 g for 20 min at 4°C and washing by centrifugation with ethanol 70% (twice), 50 μl of Tris-EDTA 0.1%, pH 7.5, were added to the pellet.

PCR method

Two different couples of primers were used for PCR analysis of the DNA from the CSF. The set of primers PEP-1 and PEP-2 described by Arthur *et al.* (5'-AGTCTTTAGGGTCTTCTACC-3', 5'-GGTGCCAACCTATGGAACAG-3') amplify the T-antigen genes (175 bp) from both BK virus (BKV) and JCV [4] (PCR 1). The set of primers JC3 and JC4 described by Henson *et al.* (5'-CAGGCTGATGAGCAAC-3', 5'-TGCAAAGAACTCCACCCTG-3') amplify the large T-antigen gene (105 bp) from JVC [5] (PCR2). The PCR mixtures (50 μl) used contained 10 μl of the extracted DNA, deoxynucleotide triphosphates at a concentration of 200 μM each, 10 μM of primers, 1 $\times$ reaction buffer (50 mM Tris-HCl, pH 8.5, 17 mM $(\text{NH}_4)_2\text{SO}_4$, 2 mM MgCl_2 , 6.7 mM EDTA, 10 mM β -mercaptoethanol, bovine serum albumin 0.1 mg/ml), gelatin 0.01% and DNA Taq polymerase (Cetus, Perkin-Elmer, Norwalk, CT, USA) 0.5 U. This was overlaid with 100 μl of mineral oil. The target DNA was amplified with 40 thermal cycles (92°C for 1 min, 55°C for 1 min, 72°C for 2 min, with a final elongation step of 72°C for 10 min) in a Hybaid thermocycler. PCR products (15 μl) were electrophoresed in an agarose 1.5% gel and visualised by exposure to UV light after staining with ethidium bromide.

Results and discussion

With the PEP-1/PEP-2 primers (PCR1), the JCV genome was detected in CSF from 10 of 11 patients with PML. CSF from one of eight patients without PML was positive for JCV by the PCR1 method (Table 1). Therefore, the positive predictive value of PCR1 was 91% and the negative predictive value was 87%.

With the JC3/JC4 primers (PCR2), CSF from all 11 patients with PML gave a positive PCR signal and of the eight AIDS patients without PML, CSF from two patients gave a positive amplification band of the appropriate size (Table 1). Therefore, PCR2 had a positive predictive value of 85% and a negative predictive value of 100%.

All results were reproduced twice and did not depend on the DNA extraction method used. However, CSF from the PML-negative patient 'Sam' gave positive

Table 1. Diagnosis and PCR findings in AIDS patients with neurological signs

Patients	Diagnosis	PCR1	PCR2
Lel	PML	+	+
Che	PML	+	+
Dup	PML	+	+
Fer	PML	+	+
Moo	PML	+	+
Aka	PML	+	+
Bri	PML	—	+
Mas	PML	+	+
Nog	PML	+	+
Aro	PML	+	+
Cla	PML	+	+
Sam	C Toxo	+	+
XFa	C Toxo	—	—
Ben	C Toxo	—	—
Dup	HIV-enceph	—	—
Lop	VZV-mening	—	+
Men	HIV-enceph	—	—
Avi	HIV-enceph	—	—
Nob	C Lymphoma	—	—

PML, progressive multifocal encephalopathy; C Toxo, cerebral toxoplasmosis; HIV-enceph, HIV encephalitis; C Lymphoma, cerebral lymphoma; VZV-mening, VZV meningitis.

results for the presence of the JCV genome by both PCR1 and PCR2 methods. In contrast, CSF from the patient 'Lop' gave a positive result for the presence of the JCV genome by the PCR2 method only and when his CSF was tested again after a period of 6 months, it gave negative results by both PCR methods. The positive PCR result for patient 'Lop' was likely to be a false positive result, while the reasons for the PCR positive result for patient 'Sam' are not clear.

CSF samples from three patients with PML were tested at different time intervals (Table 2), including after cytarabine treatment by the PCR2 method. The patients received intravenous cytarabine (2 mg/kg daily for 5 days, repeated monthly); intrathecal cytarabine (50 mg) was given once a month. The PCR signal became negative for two of the three patients during the cytarabine treatment. The PCR signal from one patient did not change during cytarabine treatment, but CSF samples were tested only once after one course of treatment, because the patient died (Table 2). However, the PCR method was not a quantitative one, as no internal standards were used, so results must be interpreted with caution.

These results, in agreement with those obtained by others, suggest that PCR is a method that may prove useful in the diagnosis of PML. However, the two different pairs of primers used did not give divergent results, as reported by others [6, 7]. Both pairs gave satisfactory results. It is noteworthy that the PCR signal was revealed by simple ethidium bromide staining. This may be due to the relatively large CSF volume (200 µl) used. Results obtained with CSF volumes larger than 100 µl correlated with the results from brain biopsy [6], but when smaller CSF volumes were used, this was not the case [5].

It has been suggested that the method of extracting DNA from the CSF may influence the outcome [6], although the two different extraction methods used in this study did not affect the results.

Digestion of the amplicons with restricted endonucleases and hybridisation with specific probes [6] were performed by others to verify the specificity of the JCV PCR. Another interesting approach for increasing confidence in the specificity of the PCR is the use of

nested primers or the sequencing of the amplicons, or both. In this study, the identification of the amplified products after PCR was performed by size determination in agarose gel and to improve the specificity of the PCR method, two sets of primers with different sequence targets were used comparatively. Corresponding PCR-positive results obtained with primers specific for BKV/JCV and primers specific for only JCV suggest that the amplicons were both of the JCV genome.

A question that must be answered is whether the PCR method detects low amounts of the viral genome and subclinical infection. Several PCR studies have shown subclinical JCV infection in the CNS and circulating B lymphocytes of HIV-infected individuals [8]. The results of the present study correlated well with the presence of clinical disease. However, CSF from one AIDS patients with cerebral toxoplasmosis ('Sam' Table 1) gave positive PCR signals with both PCR1 and PCR2 methods, whereas at necropsy no histopathological features consistent with PML were found. An increase in the CSF cell numbers secondary to toxoplasmic encephalitis could account for the PCR positivity. The detection of a subclinical infection in the CSF of this patient cannot be excluded and the necessity for a quantitative PCR should be examined.

Finally, several nucleoside analogues have been used in the treatment of PML. Therapeutic trials with cytosine arabinoside suggest that the drug is effective [9]. However, less convincing results have been reported [10]. The preliminary results presented here suggest that when PCR was used for virological monitoring during cytosine arabinoside treatment, the PCR signal of the JCV genome became negative in two patients out of three. However, the negative PCR signal was not associated with clinical improvement. Furthermore, others have reported that the PCR method showed a stable or increased JCV-DNA burden in CSF during cytosine arabinoside treatment [11].

In conclusion, the PCR method can be used for the detection of JCV in the CSF and for the diagnosis of PML. The value of PCR for monitoring treatment effects remains to be established.

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Table 2. PCR results for JCV detection in the CSF of three patients with PML, before (A) and after (B) cytarabine treatment

Patient	Date	PCR2 results
Aro	10.6.91 (A)	+
Aro	3.11.91 (B)	±
Aro	17.1.92 (B)	–
Mas	7.11.90 (A)	+
Mas	23.4.91 (B)	–
Nog	27.6.90 (A)	+
Nog	4.7.90 (B)	+

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