Ph.		Monteyne
C.J.M.		Sindic
E.C.		Laterre

Department of Neurology, Cliniques Saint-Luc, UCL, Brussels, Belgium

Recurrent Meningitis and Encephalitis Associated with Herpes simplex Type 2: Demonstration by Polymerase Chain Reaction

Dr. Ph. Monteyne, Department of Neurology, Cliniques Saint-Luc, UCL, Av. Hippocrate, 10, B-1200 Brussels (Belgium)

Pierre Mollaret described his first case of benign recurrent meningitis in 1928. About 50 years later, he wrote that nothing could be added to the original description and that the etiology remained to be discovered [1]. However, thanks to new technologies such as immu-noblotting and polymerase chain reaction (PCR) it is now sometimes possible to demonstrate the infectious origin of such syndromes. We describe a patient who developed seven episodes of meningitis and encephalitis. These episodes were found to be associated with herpes simplex type 2 (HSV 2) infection. The male patient, born in 1924, had a history of recurrent meningitis and meningoencephalitis between 1974 and 1992. In 1974, 1975 and in February 1979, he was admitted to another hospital for episodes of ‘aseptic meningitis’ with rapid recovery. He was first admitted to our center in May 1979 with fever, headache, drowsiness, and a stiff neck. There were no genital vesicles, and no history of genital herpes was reported. CT brain scan revealed only slight diffuse atrophy. Lumbar puncture showed a high protein level and 960 cells/mm³ with 59% lymphocytes (table 1). He recovered spontaneously and rapidly. In December 1988, he was admitted for a similar episode with the same symptoms in addition to aphasia and agitation. The EEG displayed abundant diffuse slow activity with anterior predominance. He recovered in 2 days, and developed another similar episode in November 1989. In December 1992, he was hospitalized with fever, aggressiveness and disorientation. His language was inadequate and the patient became confused. The EEG was diffusely slow and irregular with discharges of large slow 5- and 6-waves. As in previous episodes, he recovered rapidly. The principal results of CSF analysis are summarized in table 1. In all the samples, immunoblotting demonstrated oligoclonal IgG bands with similar patterns for both CSF and sera. Moreover, some additional CSF-restricted IgG bands were detected. An immunoaf-finity-blotting technique revealed a polyclonal pattern of anti-HSV antibodies present in both serum and CSF, also with some additional

bands that were restricted to CSF. Interestingly, specific immunoblotting was positive for all the samples analyzed (table 1). PCR detection for HSV DNA was recently performed on samples that had been kept at -20°C since 1988. Positive results were obtained on admission during the last three hospitalizations (table 1). As shown in figure 1, the PCR products had the size expected for HSV 2. All the control samples remained negative (10 normal CSF, 10 nonherpetic meningoencephalitis, 1 bacterial septicemia). As already stated, the etiology of Mollaret’s meningitis has remained unknown for many years [1]. The principal hypotheses of Pierre Mollaret were associated with an infectious origin or an immune allergic process, but he failed to isolate any infectious agent, as he was unable to produce any inflammatory process by injecting CSF from a patient with Mollaret’s meningitis to other patients either intravenously or intrathecally [1]. Since then, a few authors have suggested a herpetic viral origin in such cases. In 1982, HSV 1 was isolated from the CSF in a case of Mollaret’s meningitis [6]. Since 1991, PCR technology has allowed the detection of HSV 1 in such a case [7] while other authors have described the presence of HSV 2 [8–10]. The fact that HSV 2 DNA was more frequently amplified than HSV 1 DNA in these cases is explained by some authors as caused by a different site of latency of these two viruses [10]. However, of the ‘classical’ non-recurrent herpetic encephalitis, about 5% are due to HSV type 2 [5]. In our case, it should be stressed that there were both episodes of recurrent meningitis and bouts of clinical encephalitis. All these episodes were particularly benign with rapid recovery. To our knowledge, this is the first case of such recurrent encephalitic episodes proved to be associated with HSV 2 infection. It is not yet understood how the virus is reactivated and how it may lead to either a meningitis or an encephalitis.

Fig. 1. PCR products of CSF samples. A nested multiplex PCR procedure for the detection of both HSV 1 and HSV 2 DNA has been adapted from Aurelius et al. [4, 5] using the mentioned primers selected from within the glycoprotein D sequence of HSV 1 and from within the glycoprotein G sequence of HSV 2. After 20 cycles at 95 °C for 30 s, 57 °C for 30 s, 72 °C for 1 min, 2.5 µl of the first PCR product was reamplified for 30 similar cycles with nested primers. The products were analyzed in 2% agarose gels. The expected PCR product sizes were 138 bp for HSV 1 and 101 bp for HSV 2. Lanes 1–4: patient samples, dates as indicated; lane 5: non-inflammatory control patient; lane 6: HSV-1-positive control; lanes 7 and 8: HSV-2-positive controls; lanes 9, 10: negative PCR controls. The size is given by a 1-kb DNA ladder.

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Table 1. CSF findings

Protein mg/dl	Glucose mg/dl	Cells	Immunoblot	PCR
June 1, 1979	160	53	960	oligoclonal
June 7, 1979	129		765	oligoclonal
75% L	24% RH			59% L 34% RH
Dec. 6, 1988	450	68	1,254	oligoclonal IgG + HSV ¹ (+)
99% L	Dec. 9, 1988	260	44	819
92% L	Dec. 15, 1988	122	50	340
				oligoclonal IgG + HSV
720	99% L			100% L
oligoclonal IgG				Nov. 22, 1989
				280
				57
Aug. 26, 1992				(+)
				291
				57
660				
100% L				oligoclonal IgG + HSV
				(+)

CSF-restricted oligoclonal IgG bands were detected using an immunoaffinity-mediated capillary blot technique [2].
L = Lymphocytes; RH = ‘reticulo-hystiocytic’ cells. CSF anti-HSV antibodies were also detected by immunoaffinity blotting [3].

A time relation between PCR results and demonstration of intrathecal production of HSV antibodies has been shown in monophasic herpetic encephalitis [4]. In our case, PCR was positive on admission and became negative 3 days later in December 1988, while there was no change in immunoblotting detection in any of the samples tested, which suggested a persistent pattern of intrathecal anti-HSV antibodies. Similarly, our immunoblotting technique enabled us to detect specific intrathecal anti-HSV bands in the CSF of another patient more than 10 years after acute HSV encephalitis (unpublished data). This long-term persistence of intrathecal virus-specific antibody responses has already been reported in 9 patients studied 4.5–8 years after acute encephalitis [11].

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