

Detection of Varicella-Zoster Virus DNA by Polymerase Chain Reaction in the Cerebrospinal Fluid of Patients Suffering from Neurological Complications Associated with Chicken Pox or Herpes Zoster

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The polymerase chain reaction (PCR) was used to detect varicella-zoster virus (VZV) DNA in the cerebrospinal fluid of patients with VZV infection associated with neurological symptoms. Positive results were obtained in three of five children with post-chicken pox cerebellitis and in seven of seven herpes zoster patients with neurological symptoms. The PCR thus provides a useful tool for the early diagnosis of VZV-associated neurological disease.

Primary infection with varicella-zoster virus (VZV) and its reactivation are sometimes associated with severe neurological symptoms, such as encephalitis, meningoencephalitis, or nerve palsies (6, 7). Encephalitis following chicken pox has been assumed to be due to parainfectious events, at least in immunocompetent hosts (12). It is therefore unclear whether treatment with acyclovir (1) should be initiated in these cases.

Because VZV can only rarely be isolated from the cerebrospinal fluid (CSF) of patients with neurological disease due to herpes zoster, the isolation technique is not appropriate for proving the viral etiology of these symptoms.

We have adapted the polymerase chain reaction (PCR) technique (18) for the rapid and sensitive detection of VZV DNA sequences in CSF samples. Test sensitivity and specificity were investigated by using eight different pairs of primers from the *Xba*I M region (4) of the VZV genome, which does not contain sequence homologies with other herpesviruses (3) and also does not show genomic variation between different VZV isolates (9, 20). The primers VZ 7 (ATGTCCGTACAACATCAACT; nucleotides 3377 to 3396) (2) and VZ 8 (CGATTTTCCAAGAGAGACGC; nucleotides 3643 to 3624) yielded the highest test sensitivity for VZV-infected human fibroblasts. The amplification mixture used for VZV PCR consisted of 50 mM KCl, 10 mM Tris-Cl (pH 8.3), 2 mM MgCl₂, 200 μM of each dNTP, 10 pM of each primer, 10 μl of CSF sample (after freezing and thawing) and 2 U of *Taq* polymerase (Cetus) (18). This mixture was overlaid with paraffin, sonicated in a water bath, heat-denatured for 7 min, and subjected to 35 amplification cycles (1 min at 40°C, 1 min at 68°C, and 1 min at 94°C) in a Perkin-Elmer Cetus thermal cycler. The specificity of the amplification reaction was confirmed by slot blot hybridization as described previously (17, 19) by using as a probe the oligonucleotide VZ 9 (GGTGGAGACGACTTCAATAGC; nucleotides 3545 to 3565). To avoid contamination during the PCR procedure (14), individual steps of the assay were carried out in separate rooms, and special pipets and tips were used (13, 19).

To assess the sensitivity of this method, primary human

foreskin fibroblasts infected with VZV were tested. A concentration of 0.01 infectious units could be detected when the infected cells were diluted in double distilled H₂O (Fig. 1) or in PCR-negative CSF samples. The assay also proved to be specific for VZV DNA. No hybridization signals were observed with human DNA, with the DNA from cells infected with herpes simplex virus 1 (HSV1), HSV2, or cytomegalovirus (Fig. 1), or with 49 negative-control CSF samples (Fig. 2). The control CSFs were taken from 15 patients with other serologically proven viral infections of the central nervous system (herpes simplex encephalitis [4 cases] in the course of HSV reactivation, measles encephalitis [1 case], tick-borne encephalitis [1 case], mumps meningitis [2 cases], human immunodeficiency virus encephalopathy [4 cases], and encephalitis in the course of adenovirus infection [2 cases] and in the course of Epstein-Barr virus infection [1 case]) as well as from 34 patients with other diseases of the central nervous system (epilepsy, lumbago, multiple sclerosis, cerebral insult, neuritis, Guillain-Barre syndrome, vertigo, polyneuropathy, and meningitis and facial palsies of unknown cause). All patients were latently infected with VZV, as shown by the detection of VZV-specific immunoglobulin G (IgG) antibodies (ABs) (5) in the serum. None of these control patients, however, showed any serological evidence of VZV reactivation.

For quantitative evaluation of the PCR results, the individual bands were cut out of the filter membrane after amplification and hybridization, and the radioactivity of the bound probe was counted. The cutoff was set at the number of counts obtained with 0.05 infectious units of VZV-infected cells diluted in CSF (Fig. 2). All control CSFs yielded signals below this cutoff level.

The assay was then applied to the analysis of CSF samples from five children suffering from post-chicken pox cerebellitis, as well as from seven patients with VZV reactivation associated with neurological symptoms. The clinical diagnosis of chicken pox was serologically confirmed by the presence of virus-specific IgM ABs in the acute-phase serum sample. Laboratory confirmation of VZV reactivation was based on the presence of high complement-fixing antibody titers (≥64) (8), high levels of IgG AB (5) in both serum and CSF samples, and traces of IgM AB in the serum (four

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TABLE 1. Clinical data and PCR results obtained from CSF samples^a from five children with VZV encephalitis after the onset of chicken pox

Patient no. (age in yr)	Time of onset (days) of neurological symptoms ^b	Acyclovir therapy		PCR result at day ^b	
		Day of onset ^b	Dose	Day	PCR result
1 (6)	5			11	+
2 (5)	6	9	15 mg/kg/day	13	+
3 (6)	5	10	20 mg/kg/day	12	-
4 (6)	6	8	30 mg/kg/day	13	-
5 (5)	6	8	Not known	9	+

^a One CSF sample was obtained from each patient; contamination with erythrocytes was not observed in any of the samples.

^b Days after eruption of vesicles. Symptoms consisted of ataxia in all cases.

of the two children who received the highest dosages of acyclovir per day prior to the sampling of the CSF.

VZV DNA was detectable in the CSFs of all seven herpes zoster patients (Table 2). These results suggest the involvement of VZV in meningitis, meningoencephalitis, and palsies of cranial nerves. In contrast to virus isolation (10, 11), the PCR assay proved to be a reliable and sensitive marker for the presence of virus in the CSF and is therefore a potentially valuable tool for the early diagnosis of VZV-caused neurological disease. Positive PCR results were observed as early as day 3 after the appearance of vesicles (Table 2), several days before confirmation by serological assays is possible. While in most cases, the clinical diagnosis of VZV-caused neurological disease is facilitated by the appearance of the typical vesicular eruptions, the PCR assay is especially valuable in those cases in which no cutaneous rash can be observed (patients 2 and 4, Table 2) (10, 15). In these cases, reactivation can otherwise be confirmed only by a significant increase in AB levels, which usually takes several days. PCR will also be useful in cases where unambiguous serological diagnosis is not possible because of the presence of HSV- and VZV-cross-reactive ABs (21). Detection of VZV DNA in CSF by PCR therefore represents an early and valuable diagnostic marker in patients with neurological symptoms due to VZV.

TABLE 2. PCR results and clinical data from seven patients suffering from herpes zoster reactivation

Patient no.	Symptoms	Day of disease ^a	PCR result	Acyclovir treatment ^c
1	Meningitis	11	+	+
2	Meningitis	5 ^b	+	-
		20	-	
3	Meningoencephalitis	3	+	+
		20	-	
4	Meningitis, facial palsy	8 ^b	+	-
5	Meningitis, facial palsy	10	+	+
6	Herpes zoster neuralgia	15	+	+
7	Recurrent ophthalmic herpes zoster	21	-	-
		28	+	-
		37	-	+

^a Days after eruption of vesicles.

^b VZV reactivation indicated by serological data without eruption of vesicles. In these patients, the days of disease were counted from the onset of headache and stiffness of neck.

^c +, treatment with acyclovir; -, no treatment.

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