Detection of varicella-zoster virus DNA using the polymerase chain reaction in an immunocompromised patient with transverse myelitis secondary to herpes zoster

A D Grant, J D Fox, N S Brink, R F Miller

Abstract

A case of herpes zoster transverse myelitis is described in which the clinical diagnosis was confirmed by demonstrating the presence of varicella-zoster virus (VZV) DNA in the cerebrospinal fluid (CSF) by amplification using the polymerase chain reaction. This case illustrates the potential role of the selective amplification of VZV DNA from CSF in contributing to the diagnosis of neurological complications associated with VZV infection.

(*Genitourin Med* 1993;69:273-275)

Introduction

Although the most common neurological complication following herpes zoster is post-herpetic neuralgia, other complications may occur including oculomotor and facial nerve palsies, bladder and anal dysfunction, meningoencephalitis and myelitis.¹ Whilst an immunological pathogenesis has been suggested for certain of these neurological complications, demonstration of viral invasion of the spinal cord in a case of herpes zoster myelitis is consistent with a direct viral cytopathic effect.² We describe a case of transverse myelitis secondary to herpes zoster in a patient with AIDS that was confirmed by the detection of VZV DNA in the patient's cerebrospinal fluid (CSF) after amplification by the polymerase chain reaction (PCR).

Case report

A 41 year old heterosexual Ugandan man with no history of intravenous drug abuse was found to be HIV-1 antibody positive when he was investigated following a severe episode of *Pneumocystis carinii* pneumonia in August 1991. He was profoundly immunosuppressed with a CD4 count of $0.02 \times 10^9/l$ (normal range $0.35-2.2 \times 10^9/l$). In December of the same year he was admitted with a 3 day history of generalised headache. Neurological examination was normal apart from marked neck stiffness. Cranial magnetic resonance imaging was normal. The initial lumbar puncture performed was a traumatic tap and therefore the CSF protein was not estimated, but culture, India ink staining for cryptococci and auramine staining for acid fast bacilli were all negative. No cryptococcal antigen was detected. He was managed symptomatically, but his headache persisted and 4 days

later a repeat lumbar puncture was performed. Apart from a markedly elevated CSF protein of 4.05 g/l, the white cell count was $4/mm^3$ and the other findings were, once again, unremarkable. A presumptive diagnosis of tuberculous meningitis was made and the patient was started on rifampicin, isoniazid and pyrazinamide in conventional doses. He was not given steroids. On day 13 after admission a left sixth cranial nerve palsy was noted, and 2 days later the patient developed a flaccid paraparesis with a sensory level at the T8 dermatome and urinary retention. Magnetic resonance imaging of the spinal cord revealed an abnormal signal at the level of T5 to T8, the appearance consistent with a myelopathy (fig 1). There was no evidence of a spinal block. The following day (day 16 after admission) lesions of herpes zoster erupted bilaterally in the T7 dermatome. As the clinical appearance of the lesions, together with the dermatomal distribution were typical of herpes zoster the patient was started on acyclovir 10mg/kg intravenously 8 hourly, but despite this there was no improvement in his paraparesis. Viral culture of the CSF sample taken at the time of the development of the transverse myelitis and a further sample taken 8 days later were both negative using techniques which are known to be optimal for isolation of VZV. Both of these CSF samples were investigated for the presence of VZV DNA using the polymerase chain reaction.

The patient deteriorated further and by day 21 was confused. Further magnetic resonance imaging of his brain showed a single enhancing lesion in the right cerebellar hemisphere with surrounding oedema. In view of the possible diagnosis of cerebral toxoplasmosis sulphadiazine 2 g 8 hourly and pyrimethamine 25 mg 8 hourly were started. Despite treatment the patient had an acute neurological deterioration consistent with an intracranial haemorrhage and died 48 days after admission. A request for autopsy was refused by the family.

Nested PCR

The nested PCR method used for detection of VZV gene 29 and HSV-1 *gD* sequences has been described previously³ and was applied to the detection of cell-free virus-specific DNA in CSF samples from the patient described. The method utilises oligonucleotide primers adapted from those reported by Mahalingham *et al*⁴ and Aurelius *et al*⁵ and the detection sensitivity for VZV

Department of
Medicine and Division
of Virology,
Department of
Medical Microbiology,
University College and
Middlesex School of
Medicine, London
A D Grant
J D Fox
N S Brink
R F Miller.

Address for correspondence:
Dr J D Fox, Division of
Virology, University College
and Middlesex School of
Medicine, Windeyer
Building, 46 Cleveland
Street, London, W1P 6DB,
UK.

Accepted for publication
12 May 1993

Figure 1 T2-weighted MRI scan showing a transverse section of the thoracic spinal cord at the level of T7. The white crescent represents normal CSF and the arrow shows an area of high signal consistent with a myelopathy.

and HSV-1 after two rounds of PCR amplification was 0.01 infected cells and 0.01 infectious units, respectively.

The CSF samples were pelleted (100 000g, 2 minutes), the supernatant was boiled for 10 minutes and then cooled on ice. The first round of the nested PCR was carried out in a 50 μ l mixture containing 10mM Tris-HCl pH 8.3, 50mM KCl, 1.5mM MgCl₂, 0.01% (w/v) gelatin, 1 unit of *Taq* polymerase (Perkin Elmer Cetus, ILS Ltd., London), 200 μ M of each dNTP, 100 ng of each "outer" primer and 10 μ l of CSF supernatant. After an initial 5 minutes denaturation at 95°C, 35 cycles of 95°C for 2 minutes, 60°C for 2 minutes and 72°C for 1 minute were carried out followed by a 7 minute extension at 72°C using an MJ Research PTC-100 automated thermal cycler (GRI Ltd., Dunmow, Essex). The reaction mixture for the second round PCR was the same as for the first round except 200 ng of each "inner" primer was used instead of the "outer" primers and 1 μ l of the first round product was the source of DNA. The thermal cycling was repeated as for the first round reaction except an anneal temperature of 50°C was used and 25 cycles were found to be sufficient for detection of the second round product. Products of PCR amplifica-

tion were analysed by ethidium bromide stained agarose gel electrophoresis. Appropriate negative controls were included (both CSF and distilled water).

Cell-free VZV but not HSV-1 DNA was detected repeatedly in both CSF samples from the patient. An example of an agarose gel showing VZV-specific nested PCR products is given in fig 2.

Discussion

The presentation of a flaccid paralysis with urinary retention in this patient was clinically suggestive of a transverse myelitis. The appearance of the spinal cord on magnetic resonance imaging confirmed this diagnosis. Although a tentative diagnosis of tuberculous meningitis was made initially, this was not confirmed on repeat auramine staining and culture on three separate cerebrospinal fluid samples. Not all of the patient's neurological symptoms could, however, be attributed to his myelitis. The magnetic resonance imaging on day 21 showed a cerebellar lesion, but the demonstration of more than one type of pathological lesion in a severely immunocompromised patient is not unusual. Symptomatic myelitis following herpes zoster is a rare,¹ but well described entity. In most cases the diagnosis of transverse myelitis complicating herpes zoster is made by the observation of typical vesicular lesions in a dermatomal distribution associated with signs and symptoms suggestive of a transverse myelitis. However, transverse myelitis, as well as other neurological complications associated with herpes zoster have been described in the absence of typical skin lesions,⁶ making a clinical diagnosis impossible.

The pathogenesis of neurological complications associated with varicella-zoster virus infection remains a matter of some debate. An immunological pathogenesis has been suggested for some of these neurological complications following both primary varicella infection and herpes zoster.¹ However, in a detailed report which included post mortem examination of the spinal cord, Hogan *et al* presented evidence of direct invasion of varicella-zoster virus in a patient with transverse myelitis associated with herpes zoster.²

Varicella-zoster virus isolation from the CSF in patients with herpes zoster and transverse myelitis or other neurological complications has been described.⁷ However, this is frequently not successful, possibly because the virus is strongly cell-associated. The development of the polymerase chain reaction, a highly sensitive technique, has enabled a more precise diagnosis to be made in neurological complications associated with VZV infection. Shoji *et al*, demonstrated the presence of VZV DNA by PCR in the CSF of three of five patients with herpes zoster meningitis, one of whom did not have skin lesions. No VZV DNA was detected in the CSF of six patients with other forms of meningitis and six other controls.⁸ We have

Figure 2 VZV-specific nested PCR products analysed by agarose gel electrophoresis. Lane 1 = *phi*X174/Hae III molecular weight marker, Lane 2 = positive control equivalent to 0.01 VZV infected cells, lanes 3 and 5 = negative controls equivalent to 0.01 uninfected cells, lane 4 = VZV-specific amplification product from patient's CSF.

shown the presence of VZV DNA in the CSF of a patient with another neurological complication associated with herpes zoster, namely transverse myelitis. Although the detection of VZV DNA in the CSF from a patient with herpes zoster myelitis was compatible with the clinical and radiological diagnosis in our patient, it is not known with what frequency VZV DNA is detected in the CSF of patients with herpes zoster without neurological complications. Because VZV DNA has been detected in peripheral blood mononuclear cells taken from elderly patients,⁹ we examined cell-free supernatant of the CSF samples obtained from our patient. The demonstration of VZV sequences in the cell-free supernatant suggests that the viral DNA was intrathecal in origin. This case illustrates the potential use of the polymerase chain reaction in the diagnosis of neurological complications associated with both primary VZV infection and herpes zoster especially in cases of zoster sine herpete where the absence of the typical dermatological lesions precludes making a diagnosis by clinical association.

Dr H Kangro (Virology, St Bartholomew's Hospital) is thanked for providing VZV infected and uninfected cells for use as controls.

- 1 Gelb LD. Varicella-zoster virus. In: Virology, 2nd ed Fields BN, Knipe DM, Chanock RM, *et al.* New York: Raven Press, 1990;2011-54.
- 2 Hogan EL, Krigman MR, Hill C. Herpes zoster myelitis—evidence for viral invasion of the spinal cord. *Arch Neurol* 1973;29:309-13.
- 3 Wakefield AJ, Fox JD, Sawyerr AM, *et al.* Detection of herpes virus DNA in the large intestine of patients with ulcerative colitis and Crohn's disease using the nested polymerase chain reaction. *J Virol Methods* 1992;38:183-90.
- 4 Mahalingham R, Wellish M, Wolf W, *et al.* Latent varicella-zoster viral DNA in the human trigeminal and thoracic ganglia. *N Engl J Med* 1990;323:627-31.
- 5 Aurelius E, Johansson B, Skoldenberg B, *et al.* Rapid diagnosis of herpes simplex encephalitis by nested polymerase chain reaction of cerebrospinal fluid. *Lancet* 1991;337:189-92.
- 6 Mayo D, Booss J. Varicella zoster-associated neurologic disease without skin lesions. *Arch Neurol* 1989;46:313-5.
- 7 Heller HM, Carnevale NT, Steigbigal RT. Varicella-zoster virus transverse myelitis without a cutaneous rash. *Am J Med* 1990;88:550-1.
- 8 Shoji H, Honda Y, Murai I, *et al.* Detection of varicella-zoster virus DNA by polymerase chain reaction in cerebrospinal fluid of patients with herpes zoster meningitis. *J Neurol* 1992;239:69-70.
- 9 Gilden D, Dueland A, Devlin M, *et al.* Varicella-Zoster virus reactivation without rash. *J Infect Dis* 1992;166(Suppl 1):S30-4.