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Case Report

Chronic parvovirus B-19 meningoencephalitis with additional detection of Epstein-Barr virus DNA in the cerebrospinal fluid of an immunocompetent patient

Katrin Druschky*[,1], Judith Walloch[1], Josef Heckmann[1], Barbara Schmidt[2], Hermann Stefan[1] and Bernhard Neundörfer[1]

[1]*Department of Neurology, University of Erlangen-Nuernberg, Schwabachanlage 6, 91054 Erlangen, Germany and* [2]*Institute of Clinical and Molecular Virology, University of Erlangen-Nuernberg, Schlossgarten 4, 91054 Erlangen, Germany*

Parvovirus B19 DNA was detected by polymerase chain reaction in the brain biopsy specimen from a 67-year-old immunocompetent woman with severe chronic lymphocytic meningoencephalitis. In addition to parvovirus B19, Epstein-Barr virus DNA was identified in the CSF. Genomic material from Epstein-Barr virus was absent in the brain tissue. Clinical symptoms and CSF pleocytosis improved under long-term corticosteroid-treatment. The aetiopathogenetic role of parvovirus B19 and the possible meaning of the additionally detected Epstein-Barr virus DNA are discussed. *Journal of NeuroVirology* (2000) **6,** 418–422

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Introduction

Clinical manifestations of parvovirus B19 infection can be divided into three main categories (Heegaard and Hornsleth, 1995): (1) infection in the normal host (asymptomatic disease, erythema infectiosum, arthropathy), (2) haematologic diseases (e.g. aplastic crisis, chronic anaemia, idiopathic thrombocytic purpura), and (3) a heterogeneous group of manifestations in which the pathophysiologic role of parvovirus is not fully elucidated (neurologic, rheumatologic, and other diseases).

Most of the available publications on parvovirus B19-related neurological diseases report about meningoencephalitis and encephalopathy associated with erythema infectiosum in childhood (Balfour *et al*, 1970; Hall and Homer, 1977; Tsuji *et al*, 1990; Okumura and Ichikawa, 1993; Watanabe *et al*, 1994). In adulthood, CNS involvement by parvovirus has been reported only rarely. Heegaard *et al*. have published a case of B19 infection associated with encephalitis in a 58-year-old immunocompromised patient undergoing treatment for malignant lymphoma (Heegaard *et al*, 1995). Aseptic meningitis caused by parvovirus B19 has been described in a 26-year-old woman with sickle cell anaemia and aplastic crisis (Koduri and Naides, 1995). In previously healthy adults, parvovirus-related CNS involvement has been reported only once, so far, in a 35-year-old man with persistent B19 infection following an acute infection with meningitis (Cassinotti *et al*, 1993).

We present a second case of severe, chronic CNS infection caused by parvovirus B19 in an immunocompetent adult patient. The diagnosis was based on the detection of parvovirus DNA in a bioptically obtained brain specimen. The interesting additional finding of Epstein-Barr virus (EBV) genome in one of the collected CSF samples is discussed.

Results

Virological and microscopical examination of brain tissue

Parvovirus B19 DNA was detected by nested polymerase chain reaction (nPCR) in a biopsy specimen of brain parenchyma and meninges from a 67-year-old woman with chronic meningoencephalitis. Presence of parvovirus DNA was demon-

*Correspondence: K Druschky
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strated by three independent PCR runs. No other pathogen was found by PCR investigation of the biopsy tissue. Herpes simplex virus (HSV) was tested negative by a nested PCR which amplifies both HSV-1 and HSV-2 DNA (Yamamoto *et al*, 1991).

Microscopical examination of the brain specimen showed chronic leptomeningeal inflammation with moderate infiltration by lymphocytes. Within the cerebral cortex and white matter only sparse perivascular lymphocytic infiltrations were seen. Immunocytochemically the meningeal and intracerebral infiltrates consisted predominantly (>80%) of T cells positive for CD3. In addition, many cells in the perivascular leptomeningeal infiltrates were positive for CD20.

Virological serum and CSF studies

Serologic investigations showed positive serum IgG titers against parvovirus B19 and EBV. Parvovirus B19 antibody levels showed no significant increase when simultaneously measured by enzyme-linked immunosorbent assay (ELISA) in three stored serum samples collected on day 1, 50, and 89. No parvovirus specific IgM antibodies were detected in serum. IgG antibodies to EBV viral capsid antigen (VCA), early antigen (EA), and nuclear antigen (NA) were positive at equally low titers in the samples taken on day 1 and 68, while VCA IgM and heterophil antibodies were negative in both samples. Aside from low positive IgG titers to HSV 1, and varicella-zoster virus (VZV), serum screening revealed no further virus specific antibodies.

Table 1 gives an overview of CSF investigations on the day of admission as well as 50, 89, and 159 days thereafter. Apart from low levels of parvovirus B19 IgG, viral antibodies were negative in the CSF. Antibodies to EBV VCA were below the detection threshold of 1:10 as assessed by immunofluorescence testing. Calculation of the parvovirus-related antibody specificity index (ASI) using the approach of Reiber and Lange (1991) revealed no intrathecal humoral immune response to parvovirus (ASI < 1.5).

Parvovirus B19 DNA was positive in the CSF on day 50 (after performance of brain biopsy). Repeat CSF examination as well as the first CSF sample which had been collected on the day of admission was negative for parvovirus DNA. EBV genome was identified in the CSF obtained on day 50. Following control samples were negative for EBV DNA. No further viral genome was amplified by PCR in the collected CSF samples.

PCR examination of peripheral blood leukocytes was negative for parvovirus as well as EBV DNA.

Microbiologic assays and repeated cultures of blood and CSF did not show any bacterial, fungal, or parasitic infection. Tuberculosis was ruled out by negative PCR testing and absence of tubercle bacilli on Ziehl-Nielsen staining and 8 weeks culture on Löwenstein-Jensen medium of repeatedly obtained CSF, sputum, urine, and gastric fluid samples.

Discussion

Diagnosis of parvovirus B19-induced CNS infection was established by detection of B19 DNA in the brain tissue of an immunocompetent adult with severe chronic meningoencephalitis. Interestingly, parvovirus genome was not identified in the initial CSF sample while repeat CSF examination after biopsy revealed a positive PCR result. A possible, though speculative cause might be a release of viral

Table 1 CSF studies performed over 5 months in a 67-year-old immunocompetent patient with chronic parvovirus B19 meningoencephalitis and additional detection of EBV DNA in the CSF.

Days of hospitalisation	*1*	*50*	*89*	*128*	*159*
CSF cell count (cells/mm^3)	117	128	91	41	24
Mononuclear cells (%)	97	80	95	95	95
CSF protein (mg/dl)	1735	1750	1472	636	400
CSF/serum glucose (%)	33	26	24	30	72
CSF lactat (mmol/l)	4.4	4.9	n.d.	n.d.	4.2
Parvovirus B19 lgM[a]	neg	neg	neg	n.d.	n.d.
Parvovirus B19 lgG[a]	pos[b]	pos[b]	pos[b]	n.d.	n.d.
Parvovirus B19 DNA[c]	neg	pos	neg	neg	n.d.
EBV-anti-VCA lgG[d]	<1:10	<1:10	n.d.	n.d.	n.d.
EBV DNA	n.d.	pos	neg	neg	n.d.
Treatment	acyclovir (days 2–15), foscarnet (days 16–29), IG (days 46–50)		prednisolone (from day 55)		

[a]=tested by ELISA; [b]=positive at equally low levels; [c]=tested by nested PCR (B19 DNA was also positive in biopsy specimens of brain parenchyma and meninges – see text); [d]=tested by IF with titration; n.d.=not done; IG=immunoglobulins.

genome particles into the extracellular space by intraoperative cell damage, facilitating postoperative PCR amplification of the viral DNA.

Acute infection with parvovirus B19 was unlikely in the present case considering the patient's history of long-lasting encephalitis-related symptoms, absence of specific IgM antibodies, and absent rise in serum and CSF IgG antibody titer against parvovirus. It seems conceivable, however, that the acute B19 infection had been marked by the manifestation of a transient anaemia 2 years ago (see case report).

Calculation of the antibody specificity index (Reiber and Lange, 1991) did not indicate an intrathecal humoral immune response to parvovirus. This might be explained by the possibility of an isolated brain cell infection by parvovirus B19, resembling that of poliomyelitis-induced CNS infection in which the viral genome cannot usually be detected in CSF due to a more or less exclusive (motor) neuronal infection.

Immunosuppressive therapy with high-dose i.v. glucocorticosteroids followed by a long-term tapering course of oral prednisolone resulted in continuous clinical improvement and a marked decrease of CSF pleocytosis. We therefore assume that parainfectious immune-mediated processes were involved in the pathogenesis of parvovirus-induced brain damage.

A noteworthy aspect was the detection of a second viral pathogen in the CSF. Different possible mechanisms might account for the additional finding of EBV DNA (Pedneault *et al*, 1992): first, inflammatory CNS processes may selectively recruit lymphocytes infected with EBV; second, parvovirus B19 infection of leukocytes may activate latent EBV; third, brain cells themselves might be latently infected with EBV which may be induced into lytic replication on infection with parvovirus B19. The first two possibilities were not supported by the present PCR examinations of blood leukocytes which yielded negative results for the search of parvovirus as well as EBV DNA. Reactivation of latent brain infection with EBV was queried by the absence of EBV DNA in the patient's brain tissue. The detection of EBV DNA in the CSF might also be a casual finding and not be related to the presenting neurological symptoms at all (Portolani *et al*, 1998). In a study on acute viral encephalitis in adults, Studahl *et al* (1998) reported the detection of EBV DNA in CSF samples of four patients with HSV-1 encephalitis, in one patient with influenza A, and in another patient with encephalitis of unknown origin; the clinical significance of these findings was left unclear. According to Jeffery *et al* (1997), elevated CSF cell counts ($\geqslant 5/mm^3$) *per se* are an independent predictor of a positive PCR result.

Due to the lack of a satisfactory gold-standard test the clinical interpretation of the detected EBV DNA remains difficult. However, absence of the EBV genome in the brain tissue supports our theory that parvovirus B19 was the predominant causative pathogen in this case of chronic meningoencephalitis.

In summary, the present case is noteworthy due to the following aspects: (1) Parvovirus B19-induced CNS infection is an extremely rare event and very few adult cases have been published, so far (Heegaard *et al*, 1995; Koduri and Naides, 1995; Cassinotti *et al*, 1993). To our knowledge, this is only the second report of chronic parvovirus-related CNS involvement in an immunocompetent adult, and the first case in which presence of the viral genome was directly demonstrated in a bioptically obtained brain specimen. (2) Negative PCR testing of the CSF does obviously not exclude parvovirus B19-induced meningoencephalitis. (3) The additional detection of EBV DNA in the CSF is an intriguing finding that triggers further consideration with respect to the clinical interpretation of PCR results. (4) The effectiveness of corticosteroid-treatment implies involvement of parainfectious immune-mediated processes and provides further clarity about the underlying pathological mechanisms of parvovirus B19-related meningoencephalitis.

Materials and methods

Case report

A 67-year-old previously healthy woman was admitted to hospital due to confusion and fevers of unknown origin. For the last 6 months the patient had developed slowly progressive symptoms including weight loss, fatigue, and depression. Determination of the previous medical history revealed a transient anaemia (haemoglobin 4.9 g/dl) of unknown cause 2 years prior to the present illness. Bone marrow biopsy performed at that time had shown increased erythropoiesis and presence of large proerythroblasts.

Physical examination on admission revealed nuchal rigidity, bilaterally increased tendon reflexes, lower limb ataxia, a disturbed, clumsy gait, and a postural tremor of the hands. Routine laboratory values (chemistry, clinical haematology, and urine studies) were normal. The immunocytochemical analysis of peripheral blood lymphocytes yielded normal results apart from a slightly elevated ratio of CD4 to CD8 positive T cells. Lumbar puncture (see Table 1) showed a lymphocytic pleocytosis of 117 cells/mm^3.

MRI scan of the brain showed slight thickening of the basal meninges. Single photon emission computed tomography (SPECT) demonstrated moderate hyperperfusion of the left temporal lobe. EEG examinations showed intermittent slow-wave activity over both fronto-temporal regions.

Symptoms continuously deteriorated over the next 4 weeks with persistently elevated tempera-

tures and progressive impairment of consciousness resulting in complete unresponsiveness to verbal and tactile stimuli.

Since microbiological and virological blood and CSF assays were nondiagnostic and clinical symptoms continued to deteriorate under 'blind' treatment approaches including broad spectrum antibiotics, tuberculostatic agents, and antiviral therapy (acyclovir 10 mg/kg i.v. t.i.d. for 4 weeks, followed by foscarnet 60 mg/kg i.v. t.i.d. for 2 weeks), brain biopsy was performed on day 40 of hospitalisation. Parvovirus B19 DNA was detected in the obtained specimen by three independent PCR runs.

Following 5 days of ineffective treatment with immunoglobulins (0.4 g/kg i.v. q.d.) we started the patient on empirical immunosuppressive therapy using prednisolone at an initial dosage of 500 mg/d i.v. which was reduced to 100 mg/d p.o. after 1 week, and tapered in steps of 10 mg every 10–14 days over the following months. Within the first week of high-dose steroid treatment the patient achieved a stable level of vigilance. Continuous improvement of impaired mental and cognitive functions and of CSF pleocytosis was observed during the tapering course of prednisolone.

At follow up examination 2 months later the patient was afebrile, alert, oriented to time and place, and adequately responding to instructions on neurological examination. During the course of clinical remission there had been no recurrent attacks of fever or signs of meningeal irritation. The CSF cell count had decreased to 24/mm^3. MRI did not show any cerebral or cerebellar abnormalities apart from the small right frontal biopsy defect. The initial left temporal hyperperfusion was not apparent any more on control SPECT.

Virological studies

Serum and CSF specimens obtained on admission were tested for haemagglutination inhibition, ELISA, complement fixation and/or neutralising antibody levels to the following viruses: parvovirus B19, HSV-1, VZV, rubella, mumps, influenza A and B, cytomegalovirus (CMV), enteroviruses, adenovirus, human herpesvirus (HHV) 6, and human immunodeficiency virus (HIV) 1 and 2. Determinations of antibodies to EBV VCA, EA and NA were performed by immunofluorescence tests.

The brain tissue (1 cm^3 specimen of right frontal cortex, arachnoid layer, and dura) was analysed for genomic material of parvovirus B19, EBV, HSV-1, HSV-2, VZV, CMV, HHV 6, picorna viruses, influenza A and B, and adenovirus.

Polymerase chain reaction

CSF amplification of viral DNA was performed by centrifugation of the sample and subsequent lysis of the cellular pellet by proteinase K. Therefore, viral DNA was not amplified from cell free CSF, but from the cellular fraction of CSF.

PCR analysis of CSF as well as brain biopsy specimen and peripheral blood leucocytes for EBV DNA was performed as described by Cinque *et al* (1993). The amplification of parvovirus B19 DNA was carried out as nested PCR with the outer primers 1A (5′-AGTGGCAAATGGTGGGAAAG-3′) and 1B (5′-GGTAGTTGACGCTAACTTGC-3′) at 40 cycles of 95°C for 40 s, 55°C for 30 s and 72°C for 1 min. The inner PCR was performed under the same conditions with the primers 2A (5′-GGAACAGACTTAGAGCTTATTC-3′) and 2B (5′-GCTTGTGTAAGTCTTCACTAG-3′) as described by Clewley (1989). The resulting PCR product of 238 bp was stained with ethidiumbromide and visualised under UV light.

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