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Human herpes virus type 7 DNA in the cerebrospinal fluid of children with central nervous system diseases

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Abstract Human herpes virus type 7 (HHV-7) has been associated with unspecific febrile syndrome, exanthem subitum (ES), viral rashes and Epstein-Barr virus (EBV) like syndrome. Neurological complications such as hemiplegia or seizures have been described in a few children with ES. Whether HHV-7 may also affect the CNS in the absence of ES is unknown. In this study, we investigated CSF samples from children with different neurological diseases for the presence of HHV-7 specific DNA. A HHV-7 specific nested polymerase chain reaction (PCR) was established amplifying a 478 bp DNA sequence of the glycoprotein U23 of HHV-7 strain SB. 68 children with CNS diseases with inflammatory CSF findings (n = 24), CNS diseases without inflammatory CSF findings (n = 18) and febrile seizures (n = 26) were examined. A total of 26 children with infectious diseases in the absence of neurological disease and 11 children without signs of a peripheral infection and without neurological disease served as controls. The CSF samples of six children from the study groups were HHV-7 PCR positive, but none from the controls. These children were diagnosed with aseptic meningitis (n = 1), viral encephalitis/encephalitis (n = 2), facial palsy (n = 1), vestibular neuritis (n = 1) and febrile seizure (n = 1).

Conclusion These results indicate that human herpes virus type 7 infection is associated with central nervous system disease in children and should be considered in children whether inflammation in the cerebrospinal fluid is present or not.

Key words Central nervous system · Children · Human herpes virus type 7

Abbreviations ADEM acute disseminated encephalomyelitis · CMV cytomegalovirus · EBV Epstein-Barr virus · ES exanthem subitum · FS febrile seizure · HHV-6 human herpesvirus type 6 · HHV-7 human herpesvirus type 7 · HSV Herpes simplex virus · PBMC peripheral blood mononuclear cells

Introduction

In 1990, Frenkel et al. [13] described the isolation of a new lymphotropic herpes virus from activated CD4+ T-cells of a healthy individual. This virus was identified as a β-herpes virus closely related to human herpes virus (HHV) type 6 (HHV-6) and cytomegalovirus (CMV) [3] and designated as human herpes virus type 7 (HHV-7). Primary infection with HHV-7 occurs mainly during infancy between the ages of 2 to 5 years [5, 41]. Typically for herpes viruses, HHV-7 induces a latent infection.

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HHV-7 can be isolated from activated peripheral blood mononuclear cells (PBMC) in up to 66% of adult healthy individuals [40] suggesting that blood lymphocytes are the primary site of latency. In saliva, HHV-7 can be detected in 55%–81% of healthy adults [4, 11, 16, 42] indicating that after primary infection in childhood, viral shedding persists over months to years or may be a result of endogenous reactivation.

Serological evidence for primary HHV-7 infection has been demonstrated in children with exanthem subitum (ES) [17, 32, 33, 36] or with unspcific viral rashes [33], in one child with coincidental Epstein-Barr virus (EBV) infection [10], in a child with hepatitis [15] and in adult patients with pityriasis rosea [12]. Furthermore, HHV-7 was isolated from PBMC in child with a second event of ES [1], in a child with an EBV-like syndrome [22] and in a patient with febrile syndrome [28]. First evidence that serologically proven HHV-7 infection may cause neurological disease was provided by the reports of children with ES associated with cerebral seizures and hemiplegia [33, 34], one child with ES and encephalopathy [37] and one adult individual with facial nerve palsy [31]. Additionally, aseptic meningitis has been associated with the presence of HHV-7 in the CSF of 6/28 children [44].

The aim of this study was to investigate whether HHV-7 may be associated with inflammatory CNS disease in the absence of ES. A highly sensitive and specific nested PCR was developed to detect HHV-7 specific DNA in CSF samples. We examined the CSF samples of children with various CNS diseases and compared them with CSF samples from children diagnosed with non-neurological diseases. Here, we report that the presence of HHV-7 DNA in the CSF can be found in clinical association with inflammatory CNS disease, with cerebral nerve palsy and febrile seizures (FS).

Changes and were diagnosed with cranial nerve palsy (n = 6), hemiplegia (n = 3), cerebral seizures (n = 7) and peripheral neuropathy (n = 2). Group 3 consisted of 26 patients diagnosed with febrile seizures (FS) and had normal CSF findings. Of these patients, 22 had a first episode of FS, 5 of these were complicated. Four had a second occurrence of FS, two of which were complicated. Seizures were considered to be complicated if one or more of the following signs were present: patient younger than 6 months or older than 5 years of age; seizures lasting longer than 15 min or recurrent seizures within 24 h; focal seizures; a history of epilepsy in first degree relatives [26]. Patients with peripheral infectious diseases or non-infectious diseases, all without CNS symptoms, were used as controls. In 26 patients with various infectious diseases (control group 1) a lumbar puncture was performed to rule out CNS infection. Patients were diagnosed with pneumonia (n = 2), unspcific febrile infection (n = 6), gastroenteritis (n = 5), upper respiratory tract infection (n = 8), stomatitis (n = 1). Lyme disease stage 1 (n = 2), urinary tract infection (n = 1) and acute EBV infection (n = 1). In 11 patients with non-infectious diseases (control group 2), a lumbar puncture was performed to exclude CNS infection. Patients were diagnosed with migraine (n = 3), psychosis (n = 2), visual disturbance (n = 1), galactoamnia (n = 1), muscular hypotonia (n = 1), asphyxia (n = 1), and rheumatoid disease (n = 1). In one infant neonatal tuberculosis was excluded.

Cerebrospinal fluid samples
CSF samples were stored at –80 °C until laboratory testing. Non-human background DNA (poly A and poly T fragments of different lengths, Boehringer Mannheim, Germany) was added in a final concentration of 25 µg/ml to the CSF samples to avoid a loss of viral DNA by unspecific binding. For DNA preparation, a commercially available DNA preparation kit (QIAamp Blood Kit, Qiagen GmbH, Hilden, Germany) was used according to the manufacturer’s instructions with minor modifications. Purified DNA was eluted from the spin columns with only 50 µl “AE” buffer to achieve a higher DNA concentration in the samples. Samples with eluted DNA were stored in “AE” buffer at –20 °C until PCR analysis.

Nested polymerase chain reaction
A nested PCR was established using two pairs of new primers. The target DNA sequence was part of the genome encoding for the U23 glycoprotein of the HHV-7 strain SB [27]. The sequence of the outer pair of primers was 5'-TTGAC GACTG AACGT CGACA GTTAC-3' (25-mer; calculated annealing temperature 58.0 °C) and 5'-CACAT CAGAT ACAGA GAGTA CGGAC G-3' (26-mer; calculated annealing temperature 56.2 °C) amplifying a PCR product with a size of 755 bp. The sequence of the inner pair of primers was 5'-TTGTC TGTTG CTGTG TCC-3' (18-mer; calculated annealing temperature 45.6 °C) and 5'-AAGACCTCGA CATTTC-3' (18-mer; calculated annealing temperature 42.8 °C) producing a 478 bp DNA [6, 7, 29]. PCR conditions were: primer concentration of 0.2 µM (MWG, Munich, Germany), dNTP concentration of 0.2 µM of each nucleotide (DNA polymerisation mix, Pharmacia, Freiburg, Germany), 2.5 U Taq-polymerase (Taq polymerase gold, Perkin Elmer, USA) with a MgCl2 concentration of 4.0 mM in the first run and 3.0 mM in the second run. PCR reaction mixes were 30 µl in both runs with a sample volume of 10 µl in the first run and 1 µl in the second run. Thermal cycling conditions (Perkin Elmer Cycler 9600, Perkin Elmer, USA) were an activation step with 94 °C for 8 min followed by 45 cycles with 20 s denaturation at 95 °C, 20 s annealing at 39 °C, and 40 s synthesis at 72 °C. A final extension step with 72 °C for 8 min finished the first run. The cycler conditions of the second run differed in the number of cycles (35 cycles) and annealing temperature (51 °C for 20 s). After gel electrophoresis in an agarose gel (2%) the

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**Subjects and methods**

**Specimens**

Between 1994 and 1997, serum and CSF samples were collected after informed consent from 105 patients who had a lumbar puncture for diagnostic reasons. Routine CSF work-up included WBC, protein and glucose concentrations. CSF was considered to show inflammatory changes with a white cell count of more than 5 cells/µl and/or a protein level above 40 mg/dl. All CSF samples with an increased white cell count were tested for herpes simplex virus (HSV) type 1 and 2 by PCR to exclude HSV encephalitis. None of these samples was HSV PCR positive.

Patients with CNS diseases characterised by inflammatory CSF changes (group 1), with CNS diseases with normal CSF findings (group 2) and patients with febrile seizures (group 3) were enrolled. All patients except those with recurrent febrile seizures presented with the first episode of neurological disease. A lumbar puncture was performed during the acute onset of illness. Group 1 comprised 24 patients who were diagnosed with aseptic meningitis (n = 9), viral encephalitis (n = 8), acute disseminated encephalomyelitis (ADEM) (n = 3), Guillain-Barré syndrome (n = 3) and peripheral inflammatory neuropathy (n = 1). Group 2 consisted of 18 patients with neurological diseases in the absence of inflammatory CSF.