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Intrathecal human herpesvirus 6 antibodies in multiple sclerosis and other demyelinating diseases presenting as oligoclonal bands in cerebrospinal fluid

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ABSTRACT

Demyelinating diseases of the central nervous system (CNS) often include elevated IgG production in intrathecal space presenting as oligoclonal bands (OCBs) in cerebrospinal fluid (CSF). In most demyelinating diseases, e.g. in multiple sclerosis (MS), the underlying cause is not known. We used isoelectric focusing and affinity immunoblot to study the specificity of CSF OCBs to human herpesvirus-6 (HHV-6) in patients with demyelinating diseases of the CNS including MS. Eighty patients with positive OCB finding were included in the study. The OCBs reacted with the HHV-6 antigen in 18 cases (23%). Twelve of 46 MS patients (26%), 5 of 24 other demyelinating diseases (21%) and 1 of 10 other neurological disorders (10%) had HHV-6 specific OCBs in CSF. A specific intrathecal HHV-6 A and B antibody production was shown in a proportion of patients with demyelinating diseases and might suggest a role in the pathogenesis of these diseases.

1. Introduction

The production of intrathecal antibodies presenting as oligoclonal IgG fractions in the cerebrospinal fluid (CSF) is a common characteristic in demyelinating diseases, especially in multiple sclerosis (MS). The presence of oligoclonal fractions in infectious diseases of the central nervous system (CNS) is also well established. Indeed, oligoclonal bands (OCBs) were identified already 50 years ago in MS and subacute sclerosing panencephalitis (SSPE) (Holmoy, 2009). Since then, it has been shown that OCBs in SSPE are directed against the causative agent, i.e. the measles virus (Vandvik et al., 1976). In addition, specific antibodies to herpes simplex virus (HSV) in HSV encephalitis (Vaheri et al., 1982) and varicella zoster virus (VZV) in VZV vasculopathy (Burgoon et al., 2003) are found in the OCBs, but the specificity of OCBs in MS has remained unknown. The presence of OCBs as such in MS, however, strongly supports the potential role of infection in the pathogenesis of the disease (Gilden, 2001).

Intrathecal production of IgG in MS is evident and increased IgG index (the ratio of IgG and albumin in CSF to the IgG and albumin in serum) has been used to support the diagnosis of MS (McDonald et al., 2001; Polman et al., 2005). However, it has been stated that OCBs revealed by isoelectric focusing and immunofixation or immunoblotting are the "gold standard" with the greatest sensitivity and specificity for MS diagnosis (Andersson et al., 1994; Freedman et al., 2005). Although CSF-specific OCBs are the most relevant laboratory marker of MS, their clinical specificity is 80–90% at the most, and clinical findings together with magnetic resonance imaging (MRI) of the head and spinal cord are crucial in the diagnosis of MS. We and others have reported intrathecal antibody production against human herpesvirus 6 (HHV-6) in approximately one fifth of the patients with MS (Derfuss et al., 2005; Virtanen et al., 2007). Anyhow, it is not known if this quantitative detection of intrathecal antibodies to HHV-6 is based on polyclonal activation or if there is true clonal activation i.e. B-cell clonal expansion and activation as a result of viral exposure in the intrathecal space. Therefore, an important question is, whether OCBs in the CSF are directed against HHV-6.

In the present study we examined the specificity of OCBs for HHV-6 in a series of 80 patients with oligoclonal IgG fractions in the CSF. Altogether 18 patients, mostly patients with MS, had HHV-6-specific OCBs. The frequent detection of HHV-6-specific OCBs in the CSF of patients with MS and related disorders is a novel and intriguing finding.

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2. Patients and methods

2.1. Patients

Paired serum and CSF specimens were collected from consecutive samples and sent to the Clinical Chemistry laboratory for OCB testing by clinical consideration of the attending neurologist from Meilahti University Hospital during a one year period, from Oct 15th 2008 to Oct 15th 2009. A total of 269 samples were investigated by the routine OCB assay. Inclusion criterion for further studies was a positive OCB finding, with two or more distinct oligoclonal IgG fractions in the CSF, which do not have counterbands in serum (banding pattern types 2 and 3, Andersson et al., 1994; Freedman et al., 2005). Samples of 108 patients were positive and a total of 80 samples were obtained for this study; 28 samples were not available. The age range of the patients was 16–67 years, mean 36 years, and the ratio of females to males was 55:25. The number of oligoclonal IgG bands in the CSF ranged from 5 to 32 (mean 19.5, median 20). Samples were examined without knowing the diagnosis, and an expert neurologist settled the diagnosis blindly. Forty-six patients had a clinically definite MS according to McDonald criteria, 24 had probable demyelinating disease of the CNS (17 morbus demyelinans, 3 optic neuritis, internuclear ophthalmoplegia, diplopia, transverse myelitis, acute disseminated encephalomyelitis (ADEM)), and 10 other neurological disorders (OND) (4 meningitis, stroke, neurosarcoidosis, vertigo, spinal atrophy, epilepsy, paresthesia). The Ethics Committee of Helsinki University Central Hospital had approved the study.

2.2. Isoelectric focusing and immunofixation

Paired serum/CSF samples were diluted to the same IgG concentration (20 mg/L IgG). 10 μL of samples was isoelectrically focused using Sebia Hydragel 3 or 9 (pH gradient 3.5–9.5) CSF Isofocusing system on the Sebia Hydrasys Focusing apparatus (Sebia, Lisses, France). IgG bands were localized by performing immunofixation with peroxidase-conjugated anti-IgG antiserum and visualized with immunoperoxidase staining using reagents from Sebia.

Five different classic banding patterns have been identified in CSF diagnostics (Andersson et al., 1994; Freedman et al., 2005) and they were used in this study. In type 1, there are OCBs neither in CSF nor in serum. In type 2, OCBs are seen in CSF but not in serum. In type 3, there are some OCBs only in CSF and additional OCBs in both CSF and serum. In type 4, there are similar OCBs in CSF and serum (mirror pattern). In type 5, there are similar closely situating bands in CSF and serum indicating monoclonality (the presence of paraproteins). Of these five patterns, 2 and 3 are regarded as markers of intrathecal antibody production.

2.3. Affinity-driven immunoblot

Nitrocellulose membrane was coated with HHV-6A (Advanced Biotechnologies, Columbia, MD), HHV-6B (Meridian Life Science, Saco, ME) or HSV-1 viral antigen (purified using sucrose gradient ultracentrifugation), for 15 min at room temperature and dried for 15 min at + 37 °C. One μg of purified antigen per cm^2 of membrane was used for coating the membranes. Non-specific binding sites were blocked with 5% dry-milk. Membrane coated with only 5% milk solution was used as negative control. Isoelectrically focused serum/CSF samples in thin agarose gel were overlaid with nitrocellulose membrane coated with viral antigen. Passive transfer of proteins with one kilogram weight was allowed to proceed for 30 min. IgG bands transferred were detected with alkaline-phosphatase conjugated anti-human IgG (1:1000, DAKO, Glostrup, Denmark) and visualized using 4-nitro blue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) substrate solution. HSV-1 antigen was used as a control to ensure the specificity of the transferred IgG bands. Two CSF samples known positive for HHV-6A and two CSF samples known positive for HHV-6B were included in five consecutive runs to determine the reproducibility of the assay (Supplementary Fig. 1).

2.4. Image analysis

To quantify the bands in blots, membranes were scanned and saved as TIFF files. The intensities of bands in blots were analyzed using image analysis program IMAGEJ (U.S. National Institutes of Health, http://rsb.info.nih.gov/ij/) and presented as densitograms.

2.5. Statistical analysis

Pearson correlation test was used to test correlations between different parameters. Fisher's exact test was used to test significance between groups.

3. Results

3.1. HHV-6 specific OCBs and banding pattern

Of the 80 paired CSF-serum samples, all of which had OCBs in the CSF, altogether 18 (22.5%) showed either HHV-6A- or HHV-6B-specific bands in CSF (Fig. 1). HHV-6-specific OCBs in the CSF did not correlate with any tested parameter including the total number of OCBs in CSF,

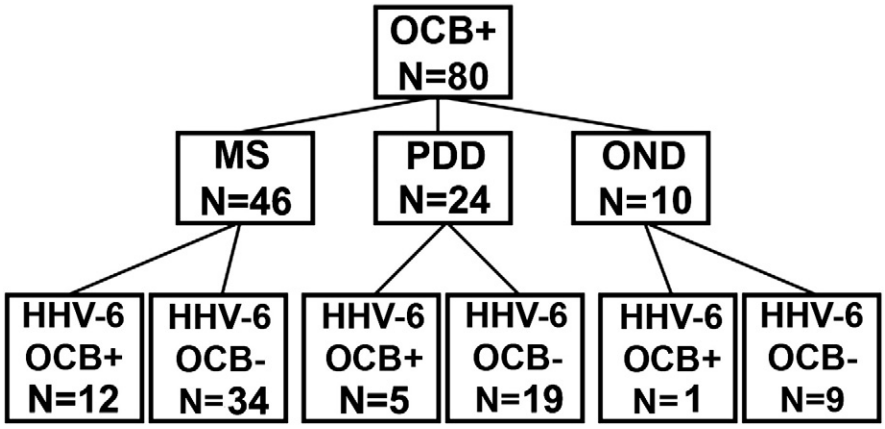

Fig. 1. Patient distribution. Patient distribution and results of HHV-6-specific OCBs in the CSF. MS, multiple sclerosis; PDD, probable demyelinating disease; OND, other neurological disease.