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Increased levels of neurotrophins in the cerebrospinal fluid of children with Epstein–Barr virus meningoencephalitis

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SUMMARY

Objective: The aim of this study was to evaluate the expression of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in the cerebrospinal fluid (CSF) of children with Epstein–Barr virus (EBV)-induced meningoencephalitis (ME) in order to establish a possible correlation with laboratory findings and neurological manifestations.
Methods: A prospective observational clinical study was performed on 10 children with viral ME, five of them with EBV-induced ME. As controls, we used CSF samples collected from children admitted with febrile seizures. Neurotrophin levels were measured using an enzyme immunoassay.
Results: Significantly higher levels of BDNF and NGF were detected in all patients with viral ME compared to controls. Moreover, in patients with EBV-induced ME, the neurotrophin levels were higher than in those with other viral ME. Of note, in children with EBV-induced ME, we found a significant correlation between neurotrophic factor levels and the number of lymphocytes in the CSF ($p < 0.001$). In these patients we also found a significant correlation between BDNF expression and the blood platelet count ($p < 0.001$). Interestingly, two patients with EBV-induced ME showed a correlation between neurotrophin increase and persistent brain abnormalities, such as prolonged alteration of mental status, psychomotor agitation, and athetosis.
Conclusions: Viral ME induces an early and strong increased biosynthesis of neurotrophic factors. This neurotrophin over-expression is likely to play a key role in the mechanisms of neuronal inflammation and in the severity of brain damage, particularly in EBV-induced ME.

1. Introduction

Epstein–Barr virus (EBV) is a member of the herpes virus family and affects B-cells and epithelial lines, leading to a variety of manifestations, ranging from subclinical infections to infectious mononucleosis, neoplasms, and severe meningoencephalitis (ME). EBV infection may also result in other neurological complications, such as cerebellitis, Guillain–Barré syndrome, seizures, and psychiatric disorders, occurring in about 6–7% of hospitalized patients.[1,2] Children with EBV-induced ME may also show metamorphopsia, the so-called 'Alice in Wonderland syndrome', in which they have perceptual distortions of personal body image, size, and spatial relationships.[3] Movement disorders indicative of extrapyramidal involvement, such as athetosis, Parkinson-like syndrome, and Sydenham-type chorea, have also been reported, while acute psychosis and transient global amnesia may be the only neurological manifestations in some cases of EBV-induced ME in children.[4–6]

Several hypotheses to explain this particular neurological virulence of EBV infection in children have been advocated, including down-regulation of type 1 interferon expression, apoptosis, and hyperinduction of proinflammatory cytokines.[7,8] It has also been reported that neurotrophin up-regulation plays a key role in the inflammatory host response after EBV-induced ME.[9,10] The neurotrophin family includes nerve growth factor (NGF) and other structurally related neuropeptides, such as brain-derived neurotrophic factor (BDNF) and neurotrophins 3/4. NGF is

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synthesized in large amounts mainly in the hippocampus and cerebral cortex. It acts on forebrain cholinergic neurons located in the septum, the nucleus of the diagonal band of Broca, and the nucleus basalis of Meynert.[11,12] The forebrain cholinergic neurons, which provide the majority of cholinergic innervation to the cerebrum and hippocampus, are particularly vulnerable in EBV-induced ME, and this may account for much of the associated memory and cognitive function impairment. BDNF is a protein consisting of 119 amino acids, produced (along with its receptor TrkB) in the hippocampus, amygdala, thalamus, and cerebellum; it exerts its biological effects on the survival and function of selected populations of dopaminergic, serotoninergic, and GABAergic neurons.[13] When neurotrophins bind to their high affinity receptors, intracellular signal channels, including mitogen-activated protein kinase (MAPK), phosphoinositide 3 (PI3) kinase, and phospholipase C-γ (PLC-γ) channels, are activated to allow manifestation of the various neurotrophin functions.[14] As it is known that NGF and BDNF are expressed locally in the hippocampus and hypothalamus of the mature brain, these neurotrophins are considered to be related to higher brain functions, such as perception, learning, and memory, exerting a key role in the formation, development, and maintenance of neuronal networks in the brain.

According to the mechanisms elicited by these neurotrophins on brain function, the objective of the present study was to investigate the expression of NGF and BDNF in the cerebrospinal fluid (CSF) of children with EBV-induced ME and to determine whether a correlation with laboratory findings and neurological manifestations exists in these patients.

2. Patients and methods

We conducted a prospective observational clinical study among children with a diagnosis of viral ME admitted to the Pediatric Infectious Disease Unit (PIDU) of our department in Rome, Italy, from January 2010 to February 2013. Children were grouped according to age, etiology of viral ME, findings on cerebral magnetic resonance imaging (MRI), clinical and laboratory characteristics, and final outcome. Ten patients were enrolled in the study: five of them had EBV-induced ME and the other five had acute viral ME due to other viral infections. As controls, we used CSF samples collected from children admitted with non-central nervous system (CNS)-related disorders, such as febrile seizures, for whom a lumbar puncture had been performed to rule out infective meningitis. Controls were matched for age and sex, respectively.

All patients underwent supportive therapy for acute viral ME. Hyperglycemia and electrolyte and metabolic derangements were strictly avoided. Fever was treated with antipyretics, such as paracetamol. Cerebral MRI was performed in all patients after the diagnosis of acute viral ME. To measure the levels of NGF and BDNF, we collected samples of CSF by lumbar puncture at admission to the hospital, before any treatment had been started. The CSF samples were submitted to microbiological and biochemical analysis (leukocyte counts, protein and glucose concentrations). CSF samples (about 1 ml) were centrifuged for 10 min at 5000 rpm and the supernatant was immediately stored at −70 °C until analysis. The outcomes of the children were assessed at discharge from the hospital using the Glasgow outcome score (GOS): a GOS of 1 was assigned to the children who died, a GOS of 2 to those in a persistent vegetative state, a GOS of 3 to those with severe neurological deficits, a GOS of 4 for mild neurological deficits, and a GOS of 5 for completely healthy children.

The study was approved by the institutional review board, and the parents of participating children were informed regarding the study and provided written informed consent.

2.1. NGF assay

NGF was quantified using a two-site immunoassay kit from Promega (USA). In brief, 96-well plates were coated with 100 μl/well of monoclonal anti-NGF antibody. After overnight incubation at 4 °C, the antibody was removed from the plates and the samples were incubated in coated wells (100 μl/well) for 6 h at room temperature. The plates were then washed 5 times with 0.05 M carbonate buffer (pH 9.5) and 1% bovine serum albumin (BSA) and the antigen was incubated overnight with polyclonal anti-human NGF antibody at 4 °C. The plates were washed again with 0.05 M carbonate buffer (pH 9.5) and 1% BSA and incubated with anti-chicken IgY horseradish peroxidase (HRP) conjugate for 2 h at room temperature. The plates were incubated with a tetramethylbenzidine (TMB)/peroxidase substrate solution for 15 min, and 1 M phosphoric acid was added (100 μl/well). The colorimetric reaction product was measured at 450 nm. NGF concentrations were interpolated from an NGF standard curve ranging from 15.6 to 1000 pg/ml of purified human NGF. The sensitivity of this assay was 3 pg/ml and cross-reactivity with other related neurotrophins was less than 5%. All assays were performed in triplicate and the NGF concentration expressed in pg/ml.

2.2. BDNF assay

The endogenous BDNF was quantified using a two-site enzyme immunoassay kit (Promega, USA). As performed for the NGF assay, 96-well immunoplates (Nunc) were coated with 100 μl/well of monoclonal anti-mouse BDNF antibody and incubated overnight at 4 °C. The plates were then washed three times with wash buffer, and the samples were incubated, with shaking, in the coated wells (100 μl each) for 2 h at room temperature. After additional washes, the antigen was incubated, with shaking, with an anti-human BDNF antibody for 2 h at room temperature. The plates were washed again with wash buffer and then incubated with an anti-IgY human purified antibody for 1 h at room temperature. After another washing, the plates were incubated with a TMB/peroxidase substrate solution for 15 min, and 1 M phosphoric acid was added to the wells (100 μl/well). The colorimetric reaction product was measured at 450 nm using an ELISA reader (Dynatech MR 5000, Germany). BDNF concentrations were determined from the regression line for the BDNF standard (ranging from 7.8 to 500 pg/ml purified mouse BDNF), incubated under similar conditions in each assay. The sensitivity of the assay was 15 pg/ml of BDNF, and the cross-reactivity with other related neurotrophins (i.e., NGF, neurotrophin 3, and neurotrophin 4/5) was less than 3%. The BDNF concentration was expressed in pg/ml for liquid samples. All assays were performed in triplicate.

2.3. EBV PCR assay

The EBV genome was extracted from the CSF samples by automated nucleic acid extraction system NucliSens EasyMAG (bioMérieux SA, Marcy l'Etoile, France) and amplified using the commercial real-time PCR kit EBV Q PCR Alert (EliTech Group, Nanogen Advance Diagnostics, Torino, Italy) and the ABI PRISM 7300 Sequencer Detection System (Applied Biosystems, CA, USA). The EBV probe, labeled with FAM fluorophor and blocked by the MGB-NFQ group, is specific for a region of the gene that codifies the Epstein–Barr nuclear antigen 1 (EBNA-1) protein of EBV. This product is able to quantify from 10 to 1 000 000 copies for the DNA of the gene coding the EBNA-1 protein of EBV per amplification reaction.

2.4. Statistical analysis

The statistical analysis of the data was performed using the StatSoft package (OK, USA), considering the experimental