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Research Report

Loss of epidermal growth factor regulation by cobalamin in multiple sclerosis

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ABSTRACT

We investigated whether the physiological regulation of cerebrospinal fluid (CSF) levels of tumor necrosis factor (TNF)-α, epidermal growth factor (EGF), and nerve growth factor (NGF) by cobalamin (Cbl) that is observed in rat and human central nervous system (CNS) is retained in the CSF of patients with multiple sclerosis (MS). The study involved 158 MS patients grouped on the basis of the different clinical courses (relapsing–remitting (RR), secondary-progressive (SP), and primary-progressive (PP)), and 76 gender- and age-matched control patients with other non-inflammatory and non-neoplastic neurological diseases. The MS patients were therapy-free at the time of lumbar puncture. CSF Cbl and EGF were blindly measured by means of radioimmunoassays, and CSF TNF-α, and NGF by means of highly sensitive enzyme-linked immunosorbent assays. Serum EGF was also measured in 38 of the MS patients and 20 healthy controls. CSF Cbl levels were significantly higher (RR patients 27.9±9.7 pg/ml, $p<0.0001$ vs. C; SP patients 25.4±8 pg/ml, $p<0.02$ vs. C), and CSF TNF-α and EGF levels significantly lower in the patients with the RR (TNF-α $28.3\pm23.4\times10^{-3}$ pg/ml, $p<0.0001$ vs. C; EGF 129.9±44.8 pg/ml, $p<0.02$ vs. C) or SP (TNF-α $20.5\pm20.5\times10^{-3}$ pg/ml, $p<0.001$ vs. C; EGF 116.5±24.8 pg/ml, $p<0.05$ vs. C) clinical course than in controls (Cbl 21±4.6 pg/ml; TNF-α $75.6\pm34.7\times10^{-3}$ pg/ml; EGF 170.2±54.8 pg/ml). There were no differences in CSF NGF or serum EGF levels between any of the MS clinical courses and controls. Our results indicate that: (a) the positive Cbl-mediated regulation of myelino- and oligodendrocyte-trophic EGF is lost in the CSF of RR- or SP-MS patients; (b) the decrease in EGF levels in the CSF may be one factor impeding CNS remyelination in MS; and (c) the PP clinical course may have different pathogenetic mechanism(s) also on the basis of the molecules investigated in this study.

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Abbreviations: BBB, blood–brain barrier; Cbl, cobalamin; Cbl-D, Cbl-deficient; CNS, central nervous system; CSF, cerebrospinal fluid; EGF, epidermal growth factor; LP, lumbar puncture; MS, multiple sclerosis; NGF, nerve growth factor; PP, primary-progressive; RR, relapsing–remitting; s, soluble; SP, secondary-progressive; TNF-α, tumor necrosis factor-α

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1. Introduction

The debate concerning the association between multiple sclerosis (MS) and vitamin B_{12} (cobalamin (Cbl)) levels in cerebrospinal fluid (CSF) is long lasting, probably because conflicting results have been obtained (reviewed in Miller et al., 2005; Scalabrino, 2005; Reynolds, 2006). Furthermore, it is thought that shifts in the CSF levels of a variety of cytokines, especially tumor necrosis factor (TNF)-α (reviewed in Chitnis and Khoury, 2003; Imitola et al., 2005; Ledeen and Chakraborty, 1998; Navikas and Link, 1996; Steinman, 2008), and some growth factors (Loeb, 2007), especially nerve growth factor (NGF) (Bracci Laudiero et al., 1992), play a role in the pathogenesis of MS. However, these studies have been limited by the fact that each study considered only one of these CSF parameters at a time and did not divide the MS patients into their different clinical courses.

We have previously acquired experimental and clinical evidence showing that Cbl regulates the levels of some cytokines and growth factors in human and rat central nervous system (CNS) and therefore seems to have non-coenzymatic functions (reviewed in Scalabrino et al., 2008). Chronic Cbl deficiency selectively increases the levels of TNF-α (Buccellato et al., 1999), NGF (Scalabrino et al., 2006), and the soluble (s) CD40:sCD40 ligand dyad (Scalabrino et al., 2008) and selectively decreases those of epidermal growth factor (EGF) (Scalabrino et al., 1999) and interleukin-6 (Scalabrino et al., 2008) in the rat CNS. Each of these abnormalities is involved in the pathogenesis of Cbl-deficient (Cbl-D) neuropathy in the rat CNS, as their correction by Cbl administration restores normal myelin ultrastructure (Scalabrino et al., 2008). Furthermore, there is an excess of TNF-α and decreased EGF levels in the CSF of patients with subacute combined degeneration (Scalabrino et al., 2004). This disease is usually included in the premortem differential diagnosis of MS even though it has not been found to be characterized by any of the primary neuropathological features of MS (Noseworthy et al., 2000).

The destruction of myelin in MS involves many non-immunological causes (Compston and Coles, 2008; Frohman et al., 2006; Lassmann et al., 2001; Trapp and Nave, 2008). It is well known that CNS astrocytes and microglia play a pivotal role in determining the CNS myelin structure because they synthesize cytokines and growth factors, some of which are myelinotrophic and some, when in excess, myelin-damaging (Kanisch, 2002; Reuss and Unsicker, 2005; Wesemann and Benveniste, 2005). Furthermore, these cytokines and growth factors can be secreted into the CSF (reviewed in Scalabrino et al., 2008). Myelin may therefore be damaged by an imbalanced glial synthesis toward a deficiency in myelinotrophic or an excess of myelin-damaging agents (Scalabrino et al., 2008). An excess of TNF-α or NGF or an EGF inactivation in rat CNS leads to myelin damage, regardless of the presence of Cbl deficiency (reviewed in Scalabrino et al., 2008).

EGF deserves particular consideration because of its positive effects on the proliferation and/or differentiation of neurons and/or oligodendrocytes (Compston et al., 1997; Dubois-Dalcq et al., 2008; Knapp and Adams, 2004). Little is known about its role in maintaining the structure and architecture of CNS myelin, but we have previously demonstrated that it is one of the key local mediators of the myelinotrophic action of Cbl in rat CNS (Scalabrino et al., 1999; Scalabrino et al., 2000).

The aim of this study was to investigate whether the Cbl-mediated regulation of TNF-α, NGF and EGF levels is maintained in the CSF of MS patients with different clinical courses. It must be emphasized that most of MS lesions are located in CNS areas which share close relationship to CSF (Tumani et al., 2009). Therefore, analysis of CSF abnormalities in MS is still valuable and useful for understanding the pathogenesis of this disease.

2. Results

CSF Cbl levels in the relapsing–remitting (RR) (27.9±9.7 pg/ml, $p<0.0001$ vs. C) and secondary-progressive (SP) (25.4±8 pg/ml, $p<0.02$ vs. C) patients were statistically significantly higher than those in the control patients (21±4.6 pg/ml); there was no statistically significant difference between the levels in the primary-progressive (PP) patients and control patients (Fig. 1).

CSF TNF-α levels in the RR ($28.3\pm23.4\times10^{-3}$ pg/ml, $p<0.0001$ vs. C) and SP ($20.5\pm20.5\times10^{-3}$ pg/ml, $p<0.001$ vs. C) patients were significantly lower than those in the control patients ($75.6\pm34.7\times10^{-3}$ pg/ml); there was no statistically significant difference between the PP patients and control patients (Fig. 2). In the RR patients, there was a significant negative correlation ($r=-0.7866$; $p<0.05$) between TNF-α and Cbl levels.

CSF EGF levels were significantly lower in the RR (129.9±44.8 pg/ml, $p<0.02$ vs. C) and SP (116.5±24.8 pg/ml, $p<0.05$ vs. C) (but not in the PP) patients than in the control patients (170.2±54.8 pg/ml) (Fig. 3). Interestingly, there were no statistically significant differences in serum EGF levels between any of the MS clinical courses and the healthy controls (Fig. 4).

There were no statistically significant differences in CSF NGF levels between any of the MS clinical courses and the control patients (Fig. 5).

Lastly, it should be noted that most of the control values of each CSF parameter tested is well distributed within the confidence limits of the mean, although the control patients were affected by different non-inflammatory and non-neoplastic neurological diseases.

3. Discussion

Our study relied on two different lines of evidence: we found abnormal CSF levels of Cbl, TNF-α and EGF in the RR and SP patients, and the loss of the Cbl-mediated regulation of CSF EGF and NGF levels in the same two MS clinical courses.

Given that MS is an extremely heterogeneous disease with respect to clinical course and pathogenetic mechanisms (Tumani et al., 2009), we considered mandatory to divide the MS patients into their different clinical courses. This had the negative draw-back that the numbers of MS patients assigned to the different clinical courses were not equalized because of the different frequency of the MS clinical courses.

Our results demonstrate that CSF Cbl levels are increased in MS patients with the RR and SP clinical courses, but unchanged in those with the PP clinical course. This disagrees with the