Neuroscience 298 (2015) 293–301

LOW LEVELS OF COBALAMIN, EPIDERMAL GROWTH FACTOR, AND NORMAL PRIONS IN MULTIPLE SCLEROSIS SPINAL CORD

G. SCALABRINO, [a]* **D. VEBER,** [a] **R. DE GIUSEPPE** [b] **AND F. RONCAROLI** [c]

[a] *Department of Biomedical Sciences, Laboratory of Neuropathology, University of Milan, 20133 Milan, Italy*

[b] *Department of Biomedical, Surgical and Dental Sciences, University of Milan, 20122 Milan, Italy*

[c] *Division of Brain Sciences, Imperial College, London W12 0NN, UK*

Abstract—We have previously demonstrated that multiple sclerosis (MS) patients have abnormal cerebrospinal fluid (CSF) levels of the key myelin-related molecules cobalamin (Cbl), epidermal growth factor (EGF), and normal cellular prions (PrP^Cs), thus confirming that some CSF abnormalities may be co-responsible for remyelination failure. We determined the levels of these three molecules in post-mortem spinal cord (SC) samples taken from MS patients and control patients. The control SC samples, almost all of which came from non-neurological patients, did not show any microscopic lesions of any type. All of the samples were supplied by the U.K. MS Tissue Bank. The Cbl, EGF, and PrP^C levels were determined using enzyme-linked immunosorbent assays. The SC total homocysteine level was determined using a competitive immunoenzymatic assay. CSF samples, taken from a further group of MS patients, were used for the assay of holo-transcobalamin (holo-TC) levels. The Cbl, EGF, and PrP^C levels were significantly decreased in MS SCs in comparison with controls and, paradoxically, the decreased Cbl levels were associated with decreased SC levels of homocysteine, a biochemical marker of Cbl deficiency. The trends of EGF and PrP^C levels paralleled those previously found in CSF, whereas that of Cbl was the opposite. There was no significant difference in CSF holo-TC levels between the MS patients and the controls. Given that we have previously demonstrated that Cbl positively regulates central nervous system EGF levels, it is conceivable that the low EGF levels in the MS SC may be causally related to a local decrease in Cbl levels. Only PrP^C levels were invariably decreased in both the SC and CSF regardless of the clinical course of the disease. These findings suggest that the simultaneous lack of Cbl, EGF, and PrP^Cs may greatly hamper the remyelination process in MS patients, because they are key molecules of the machinery for remyelination.

Key words: cobalamin, epidermal growth factor, holo-transcobalamin, multiple sclerosis, normal prions, spinal cord.

INTRODUCTION

It is widely recognized that cobalamin (Cbl), epidermal growth factor (EGF), and normal cellular prions (PrP^Cs) are relevant to central nervous system (CNS) myelin maintenance (Scalabrino et al., 2014), although none of them directly controls CNS myelin synthesis (Plata-Salamán, 1991; Prusiner, 1998, 2001; Linden et al., 2008; Scalabrino, 2009). We have previously identified some non-canonical (i.e. non-coenzymic) Cbl functions related to its myelinotrophic properties: (i) it stimulates the synthesis and/or increases the levels of the myelinotrophic EGF and interleukin-6, and decreases the synthesis and/or levels of the potentially myelinotoxic nerve growth factor and tumor necrosis factor-α in the rat CNS and human cerebrospinal fluid (CSF), thus shifting the balance in favor of the myelinotrophic molecules (Scalabrino, 2009); (ii) it up-regulates the levels of pan-neurotrophin receptor p75 (Twiss et al., 2006) in the rat spinal cord (SC) (Scalabrino, 2009); and (iii) it down-regulates the PrP^C levels in the human CSF and rat SC (Scalabrino et al., 2012, 2013). It has been shown that the EGF is as effective as Cbl in ''curing'' the SC myelin lesions of Cbl-deficient (Cbl-D) rats without modifying their Cbl-D status (Scalabrino et al., 2000), stimulates oligodendrocyte (ODC) progenitor differentiation and maturation *in vitro* (Compston et al., 1997; Chandran et al., 1998; Knapp and Adams, 2004), and induces the subventricular zone type B cells to differentiate into ODCs (Gonzalez-Perez et al., 2009). The EGF effect on the subventricular zone cells is relevant to multiple sclerosis (MS), because this zone has been shown to be activated in the MS brain (Nait-Oumesmar et al., 2007). It is worth remembering that two myelinotrophic agents (Cbl and EGF) stimulate *in vivo* PrP^C synthesis in the SC of Cbl-D rats (Scalabrino et al., 2012). Finally, the strict relationship between PrP levels and

*Corresponding author. Address: Department of Biomedical Sciences, Laboratory of Neuropathology, University of Milan, Via Mangiagalli, 31, 20133 Milano, Italy. Tel: +39-0250315348; fax: +39-0250315338.

E-mail address: giuseppe.scalabrino@unimi.it (G. Scalabrino).

Abbreviations: Abs, antibodies; AIA, automated immunoassay analyzer; Cbl, cobalamin; Cbl-D, Cbl-deficient; CNS, central nervous system; CSF, cerebrospinal fluid; EGF, epidermal growth factor; ELISA, enzyme-linked immunosorbent assay; holo-TC, holotranscobalamin; LFB, Luxol Fast Blue; MHC, major histocompatibility complex; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; ODC, oligodendrocyte; ORO, Oil Red O; PK, proteinase K; PP, primary-progressive; PrP^C, normal cellular prion; PrP^{SC}, PrP scrapie; r.p.m., revolutions per minute; RR, relapsing-remitting; SC, spinal cord; SP, secondary-progressive; tHCYS, total homocysteine.

http://dx.doi.org/10.1016/j.neuroscience.2015.04.020

CNS myelin status was first highlighted by neuropathological studies of human PrP diseases, and subsequently in the CNS of PrP^C knock-out mice or mice overexpressing PrP^C or different parts of the PrP^C molecule (for reviews, see: Prusiner, 2001; Linden et al., 2008; Martins et al., 2010).

We have previously demonstrated that the positive Cbl regulation of EGF levels is lost in the CSF of patients with relapsing-remitting (RR) or secondary-progressive (SP) MS insofar as they have significantly higher Cbl levels and significantly lower EGF levels than controls, but no changes in serum EGF levels have been found (Scalabrino et al., 2010). We have also recently demonstrated that CSF PrP^C levels are significantly lower in patients with RR or primary-progressive (PP) MS than in controls, but not in patients with Alzheimer's disease or amyotrophic lateral sclerosis (Scalabrino et al., 2013; Scalabrino and Veber, 2014).

Much research has focused on demyelination and remyelination in MS, because the former is deemed to be a key neuropathological hallmark, and the latter a beneficial process that also influences the clinical course of the disease (Lucchinetti et al., 2000; Irvine and Blakemore, 2008; Goldschmidt et al., 2009; Bramow et al., 2010; Franklin and Gallo, 2014). Furthermore, incomplete SC remyelination correlates with a more severe MS course (Bramow et al., 2010). However, it has been shown that remyelination varies widely in MS patients, being widespread in some and sparse in others (Patrikios et al., 2006; Patani et al., 2007). Nevertheless, it is generally agreed that remyelination is a transient phenomenon because it eventually fails, especially in the end-stage of the disease (Hohlfeld, 2002; Miller and Mi, 2007; Franklin and ffrench-Constant, 2008; Reynolds et al., 2011; Franklin et al., 2012; Lassmann, 2014). The mechanisms that doom remyelination to failure are still unclear, but include the loss and/or destruction of ODC progenitors, the lack or dysregulation of gliotrophic and/or neurotrophic factors, the loss or abnormal composition of axons, and the excess of some myelination-inhibiting molecules (Franklin and Hinks, 1999; Niehaus et al., 2000; Wolswijk, 2000, 2002; Chang et al., 2002; Franklin, 2002; Rosenberg et al., 2006; Kuhlmann et al., 2008).

On the basis of all the above, we determined the Cbl, EGF, and PrP^C levels in autoptic SC samples obtained from MS patients in order to verify their possible local absence and ascertain whether their levels parallel those previously found in MS CSF (Scalabrino et al., 2010, 2013). Lastly, given the unexpected findings of decreased Cbl levels in MS SCs (see below), we investigated Cbl status more thoroughly by determining: (i) SC levels of total homocysteine (tHCYS), a well known biochemical marker of Cbl deficiency (Scalabrino, 2009); and (ii) CSF holo-transcobalamin (holo-TC) levels because holo-TC binds biologically active Cbl (Herrmann and Obeid, 2007; Schrempf et al., 2011).

EXPERIMENTAL PROCEDURES

Clinical characteristics of controls and MS patients from whom the SC samples were taken, and post-mortem SC samples

Post-mortem SC samples came from 16 patients with clinically and neuropathologically confirmed MS (see Table 1) and 13 controls (eight males and five females; mean age 78.1 years, range 53–91 years) who died of non-neurological diseases (12 cases) or a non-MS neurological disease (one case). We chose non-neurological patients as controls in order to be as sure as possible that their SCs would be devoid of any microscopic pathological findings. All of the samples were supplied by the U.K. MS Tissue Bank at Imperial College, London. The inclusion criteria for the MS patients were: (i) a clinical diagnosis of MS; (ii) a neuropathological diagnosis of MS; and (iii) no clinical and/or neuropathological evidence of a CNS neoplasm or non-MS and non-demyelinating disease, a CNS vascular accident, or a virus-induced CNS demyelinating disease. Detailed clinical assessment was available in all cases including patients' age at the time of disease onset, the number and nature of clinical relapses, an estimate of the time to conversion to secondary progression, the clinical course, the time at which the patient required the use of a wheelchair, total disease duration, and age at death.

All of the SC samples were collected with the fully informed consent of the donors and their next-of-kin. The procedures for sample retrieval, processing and storage received the approval of the Ethics Committees of Imperial College of London and Department of Biomedical Sciences of University of Milan. The MS

Table 1. Clinical data of multiple sclerosis (MS) patients from whom the spinal cord samples were taken.

Patient No.	Gender	Clinical course	Disease duration (years)	Age at death (years)
1	F	SP	43	73
2	F	SP	36	64
3	F	SP	23	49
4	F	SP	35	71
5	F	PP	6	42
6	F	SP	21	77
7	F	SP	26	53
8	F	RR	25	46
9	F	SP	13	58
10	M	PP	29	66
11	F	SP	29	48
12	M	SP	18	43
13	M	SP	16	40
14	F	SP	11	42
15	F	SP	27	55
16	M	SP	10	39
Mean value ± SD			23 ± 10.3	54.1 ± 12.6

F = female; M = male; PP = primary-progressive; RR = relapsing-remitting; SD = standard deviation; SP = secondary-progressive.