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Mini-review

Cobalamin and normal prions: A new horizon for cobalamin neurotrophism

Giuseppe Scalabrino*, Daniela Veber

"Città Studi" Department, Laboratory of Neuropathology, University of Milan, via Mangiagalli 31, 20133 Milan, Italy

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ABSTRACT

It is known that cobalamin (Cbl) deficiency damages myelin by increasing tumor necrosis factor (TNF)-α and decreasing epidermal growth factor (EGF) levels in rat central nervous system (CNS), and affects the peripheral nervous system (PNS) morphologically and functionally. It is also known that some polyneuropathies not due to Cbl deficiency are connected with increased TNF-α levels, and that various cytokines (including TNF-α) and growth factors regulate the *in vitro* synthesis of normal prions (PrP^Cs). Given that there is extensive evidence that PrP^Cs play a key role in the maintenance of CNS and PNS myelin, we investigated whether the PrP^C octapeptide repeat (OR) region is involved in the pathogenesis of rat Cbl-deficient (Cbl-D) polyneuropathy. After intracerebroventricularly administering antibodies (Abs) against the OR region (OR-Abs) to Cbl-D rats to prevent myelin damage and maximum nerve conduction velocity (MNCV) abnormalities, and PrP^Cs to otherwise normal rats to reproduce PNS Cbl-D-like lesions, we measured PrP^C levels and MNCV of the sciatic and tibial nerves. PrP^C and TNF-α levels were increased in sciatic and tibial nerves of Cbl-D and saline-treated rats, and the OR-Abs normalized the myelin ultrastructure, TNF-α levels, and MNCV values of the sciatic and tibial nerves of Cbl-D rats. The same peripheral nerves of the otherwise normal PrP^C-treated rats showed typical Cbl-D myelin lesions, significantly increased TNF-α levels, and significantly decreased MNCV values. These findings demonstrate that Cbl deficiency induces excess PrP^Cs and thereby excess OR regions, which seem to be responsible for the PNS myelin damage, as has recently been found in the case of CNS myelin damage [66]. Furthermore, excess TNF-α is also involved in the pathogenesis of Cbl-D polyneuropathy. In conclusion, we have extended the list of prion diseases by adding one caused by excess PrP^Cs and the polyneuropathies related to excess TNF-α.

1. Introduction

In mammalian cells there are only two known cobalamin (Cbl)-dependent enzymes: (*i*) L-methylmalonyl-coenzyme A (CoA) mutase (EC 5.4.99.2) requires adenosyl-Cbl and catalyzes the conversion of L-methylmalonyl-CoA to succinyl-CoA; and (*ii*) methionine synthase (EC 2.1.1.13) requires methyl-Cbl and catalyzes the simultaneous conversion of N^5-methyltetrahydrofolate to tetrahydrofolate and of homocysteine (HCYS) to methionine [1]. Therefore, the metabolites methylmalonic acid (MMA) and HCYS accumulate when these two enzymatic reactions are impaired by Cbl deficiency [1].

Cbl-deficient (Cbl-D) neuropathy is generally due to: (*i*) defective Cbl absorption because of intrinsic factor failure, as in the case of pernicious anaemia; (*ii*) reduced dietary intake, such as in a strict vegetarian diet; and (*iii*) partial or total gastrectomy. Cbl deficiency frequently occurs in the elderly and may go unrecognized because of its subtle clinical manifestations [2,3]. Furthermore, some studies have drawn much attention to the often unappreciated fact that some Cbl-D patients may have serious neurological dysfunction, even when they have no recognizable hematological abnormalities [4,5].

Classically, peripheral neuropathy is one of the main neuropathological consequences of acquired cobalamin Cbl deficiency in adults [6–8]. Cbl deficiency causes neuropathological and ultrastructural hallmarks (i.e.: intramyelinic and interstitial edema, and glial activation [8,9]) in the peripheral nervous system (PNS) and central nervous system (CNS) concomitantly with electrophysiological

Abbreviations: Abs, antibodies; Cbl, cobalamin; Cbl-D, Cbl-deficient; CNS, central nervous system; CoA, coenzyme A; CSF, cerebrospinal fluid; EGF, epidermal growth factor; HCYS, homocysteine; hi, heat-inactivated; MMA, methylmalonic acid; MNCV, maximum nerve conduction velocity; mdr, multidrug resistance; NGF, nerve growth factor; ON, otherwise normal; OR, octapeptide repeat domain of the normal prion molecule; PNS, peripheral nervous system; PrP^C(s), normal prion(s); *r*, recombinant; (SAM), *S*-adenosyl-L-methionine; SC, spinal cord; TGX, totally gastrectomized; TNF, tumor necrosis factor.

* Corresponding author. Tel.: +39 02 50315348; fax: +39 02 50315338.
E-mail address: giuseppe.scalabrino@unimi.it (G. Scalabrino).

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PNS abnormalities [6–11]. We have previously reproduced all of the morphological hallmarks of human acquired Cbl-D neuropathy in the PNS and CNS of rats made Cbl-D by means of total gastrectomy or a Cbl-D diet, and also provided evidence of electrophysiological PNS damage [12]. Understanding the pathogenesis of Cbl-D neuropathy is of great interest and may be of paramount importance because Cbl deficiency is fairly common in humans. A number of reviews by other authors [1,13–15] and ourselves [9,10,16] is available concerning various aspects of Cb-D neuropathology.

Classical hypotheses concerning the pathogenesis of Cbl-D neuropathy have postulated a casual relationship between Cbl-D neuropathy and the impairment of either of the two mammalian Cbl-dependent enzymes, which leads to MMA and HCYS accumulation and a decrease in *S*-adenosyl-L-methionine (SAM) supply [10]. MMA and HCYS have been viewed as putative "neurotoxins" not only in the case of Cbl-D neuropathy [17,18], but also as being responsible for spongy vacuolation in a Cbl-D CNS [13]. These hypotheses have been discussed extensively elsewhere [1,13–16,19].

We demonstrated that the severity of the neuropathological features in the spinal cord (SC) of Cbl-D rats does not correlate with the accumulation of MMA and HCYS in their sera and SC [20]: no substantial increase in the severity of Cbl-deficiency-induced lesions in the SC white matter was observed as the time of Cbl deficiency lengthened, making it unlikely that the accumulation of the putative "neurotoxins" (see above) is responsible for the Cbl-deficiency-induced lesions in the SC white matter of Cbl-D rats [20].

We have identified new pathogenetic mechanisms underlying Cbl-D neuropathy in the CNS (especially the SC) of adult rats by demonstrating that the lesions are caused not by mere Cbl withdrawal but by concurrent abnormalities in the CNS synthesis and cerebrospinal fluid (CSF) levels of some cytokines and growth factors [9,10]. These abnormalities include an increase in some myelinotoxic agents (e.g. tumor necrosis factor (TNF)-α [9,10] and nerve growth factor (NGF) [9,10]) and a decrease in some myelinotrophic agents (e.g. epidermal growth factor (EGF) [9,10] and interleukin-6 [9,10]). It must be emphasized that increased CNS TNF-α levels of Cbl-D rats are not concomitant with any morphological evidence of local inflammation, thus highlighting the pure myelinotoxic property of excess TNF-α [9,10]. Interestingly, the negative regulation of NGF synthesis by Cbl has been indirectly confirmed by Battaglia-Hsu et al. [21] who demonstrated that neuroblastoma cells transfected with a chimeric Cbl-binding protein, transcobalamin-oleosin, and thereby showing an intracellular Cbl sequestration, showed increased pro-NGF levels.

The importance of SAM as neuroprotective agent is emphasized by the findings that: (*i*) SAM treatment to Cbl-D rats substantially reduced the severity of the SC white matter lesions and significantly decreased the CSF levels of soluble CD40 ligand (a cell-surface protein belonging to the TNF family) [22]; and (*ii*) SAM treatment of neuroblastoma cells cultured in a vitamin B-deficient medium restores normal gene methylation levels of presenilin-1, previously decreased by vitamin B deficiency [23].

The changes in the cytokine and growth factor network of rat CNS is etiologically related to Cbl-D status, as it can be substantially corrected by Cbl treatment in Cbl-D rats, together with the ultrastructural lesions of their SC white matter [9,10,12]. Furthermore, the presence of the same abnormalities in the totally gastrectomized (TGX) rats and the rats fed the Cbl-D diet leads us to exclude the possibility that the CNS abnormalities in the TGX rats may be due to malabsorption and/or hormonal derangements following total gastrectomy [16], also because the electrophysiological abnormalities and neuropathological changes concurrent with a denutrition status (the so-called "malnutrition neuropathy" [12]) are totally different from those due to Cbl deficiency, however the vitamin deficiency had been induced.

No histological signs of apoptosis were observed in any SC region of Cbl-D rats, despite reports of apoptosis have been reported by others in the CNS of TNF-α-treated rats [24,25], in cultured Cbl-deprived neoplastic cells [26], and, more recently, in cultured neuroblastoma cells transfected with plasmid containing transcobalamin-oleosin, a very efficient chelator of Cbl [27].

Normal cellular prion protein (PrP^C), a glycophosphatidylinositol-anchored syaloglycoprotein encoded by the Prnp gene, is widely expressed in eukaryotic cells and mainly located at the plasma membrane [28–31]. Many different neurological and other functions have been assigned to PrP^C, including the maintenance of PNS and CNS myelinated fibers, synaptic transduction, signal transduction, neuroprotection against some CNS injuries, and copper binding [32–39]. PrP^C is also present in plasma [40] and CSF [41,42], although it is still unclear whether the soluble and membrane-bound forms are identical.

It is worth recalling here that PrP^Cs play a crucial role in maintaining normal myelin structure in the CNS and PNS of mice [43]. Although studies of mice expressing only some PrP^C portions have made it possible to identify definite PrP^C domains with different functions [30,44], the functions of PrP^Cs in the CNS and, particularly, in the PNS are still largely unknown. It has been shown that PrP^C-deficient mice develop a late-onset polyneuropathy that has the typical morphological features of a demyelinating peripheral neuropathy that can be prevented by the genetic reintroduction of PrP^C expression [43], and that transgenic mice which overexpressing PrP^C develop a demyelinating peripheral neuropathy together with CNS vacuolation [45]. All of these findings suggest that PrP^Cs may be neuroprotective in some cases and neurotoxic in others [46–51]. Furthermore, little is known about the regulation of PrP^C synthesis, but it has been shown that it can be modulated by TNF-α [31,52,53], NGF [31,53–55] and EGF *in vitro* [52], whereas the role of nuclear factor-κB is still uncertain [56–58].

We postulated that PrP^Cs may be involved in the pathogenesis of the peripheral neuropathy observed in Cbl-D rats because: (*i*) they are normally expressed by Schwann cells [59,60]; (*ii*) TNF-α is a key molecule involved in both PrP^C synthesis [31,52,53] and the pathogenesis of Cbl-D central neuropathy [9,10]; (*iii*) the PNS of Cbl-D rats is characterized by morphological abnormalities in Schwann cells [12], which normally express PrP^C [61]; and (*iv*) reactive gliosis and spongy vacuolation, together with a substantially normal axon morphology, are common features of rat Cbl-D neuropathy [9,10,12] and PNS of some PrP^C-deficient or PrP^C-overexpressing mice [30,43,45,62,63]. Therefore, this article will mainly concentrate on the new perspectives concerning the pathogenesis of Cbl-D neuropathy based on the link between Cbl and PrP^C recently identified in our laboratory.

To test this hypothesis, we repeatedly injected anti-PrP^C antibodies (Abs) [64] into the CSF of TGX rats with the aim of preventing the onset of the typical lesions of Cbl-deficiency-induced peripheral neuropathy. The same Abs were repeatedly administered to rats chronically fed a Cbl-D diet as a counter-proof of their specific link to the effects of Cbl deficiency. We chose Abs whose epitope is the 58–88 aa octapeptide repeat (OR) region of the PrP^C molecule (OR-Abs) [64] because this is considered to be an important domain for PrP^C functions [65] (even though it has not been demonstrated to be required for myelin maintenance) and, for comparative purposes, Abs whose epitope is a portion of the 142–156 aa C terminus region of the PrP^C molecule [64], and subsequently determined PrP^C levels in the sciatic and tibial nerves of the treated Cbl-D rats. We also repeatedly injected recombinant (*r*) mouse PrP^Cs into the CSF of otherwise normal (ON) rats in order to investigate whether the excess reproduced the key neuropathological lesions of Cbl-D peripheral neuropathy, because this would further support our hypothesis. We then measured TNF-α