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Review article

Are PrP^Cs involved in some human myelin diseases? Relating experimental studies to human pathology

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ABSTRACT

We have experimentally demonstrated that cobalamin (Cbl) deficiency increases normal cellular prion (PrP^C) levels in rat spinal cord (SC) and cerebrospinal fluid (CSF), and decreases PrP^C-mRNA levels in rat SC. Repeated intracerebroventricular administrations of anti-octapeptide repeat-PrP^C-region antibodies to Cbl-deficient (Cbl-D) rats prevent SC myelin lesions, and the administrations of PrP^Cs to otherwise normal rats cause SC white matter lesions similar to those induced by Cbl deficiency. Cbl positively regulates SC PrP^C synthesis in rat by stimulating the local synthesis of epidermal growth factor (EGF), which also induces the local synthesis of PrP^C-mRNAs, and downregulating the local synthesis of tumor necrosis factor(TNF)-α, thus preventing local PrP^C overproduction. We have clinically demonstrated that PrP^C levels are increased in the CSF of patients with subacute combined degeneration (SCD), unchanged in the CSF of patients with Alzheimer's disease and amyotrophic lateral sclerosis, and decreased in the CSF and SC of patients with multiple sclerosis (MS), regardless of its clinical course. We conclude that SCD (human and experimental) is a neurological disease due to excess PrP^C without conformational change and aggregation, that the increase in PrP^C levels in SCD and Cbl-D polyneuropathy and their decrease in MS CNS make them antipodian myelin diseases in terms of quantitative PrP^C abnormalities, and that these abnormalities are related to myelin damage in the former, and impede myelin repair in the latter.

Contents

Abbreviations: Abs, antibodies; Akt, cytoplasmic serine–threonine kinase from Ak mouse strain thymoma; AT, anti-TNF-α; Cbl, cobalamin; Cbl-D, Cbl-deficient; CNS, central nervous system; CoA, coenzyme A; CSF, cerebrospinal fluid; EGF, epidermal growth factor; GFAP, glial fibrillary acidic protein; HCYS, homocysteine; i.c.v., intracerebroventricular; IL, interleukin; KO, knock-out; L, ligand; MMA, methylmalonic acid; MNCV, maximum nerve conduction velocity; MS, multiple sclerosis; NGF, nerve growth factor; ODC, oligodendrocyte; ON, otherwise normal; OR, octapeptide repeat; PNS, peripheral nervous system; PA, pernicious anemia; PrP^C, normal cellular prion; PrP^{SC}, PrP scrapie; s, soluble; SAM, *S*-adenosyl-*L*-methionine; SC, spinal cord; SCD, subacute combined degeneration; Tg, transgenic; TGF, transforming growth factor; TNF, tumor necrosis factor.

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1. Introductory remarks concerning the key role of the normal cellular prions (PrP^Cs) and cobalamin (Cbl) in myelin maintenance in the central (CNS) and peripheral nervous system (PNS)

1.1. PrP^Cs

Important evidence of the role of PrP^Cs in myelin maintenance comes from studies of PrP^C knock-out (KO) mice and/or mice lacking one or more parts of the PrP^C molecule [1–4]. Although PrP^C KO mice display no overt neural phenotype, a variety of subtle CNS phenotypes (including spinal cord (SC) myelin damage) have been reported in these mouse strains [5–7], and late-onset polyneuropathy has been observed in different types of PrP^C KO mice [7].

Mice expressing PrP^C molecules deleted of different portions die neonatally [8,9]. Some strains of transgenic (Tg) mice overexpress some parts of the PrP^C molecule [8,9]. Tg mice expressing the PrP^C gene (*Prnp*) with point mutations, insertions or deletions develop a spectrum of neuropathological pictures reminiscent of those associated with transmissible spongiform encephalopathies [9–11]. Tg mice overexpressing wild-type PrP^Cs or expressing PrP mutants with the deletion of different PrP^C regions show severe myelin lesions of the SC and/or brain [12]. Other strains of Tg mice expressing PrP mutants with PrP^C deletions show severe PNS myelin damage [7]. Oligodendrocyte (ODC)-restricted PrP^C expression selectively suppresses PrP scrapie(PrP^{SC})-induced leukoencephalopathy and prolongs mouse survival [11,13].

The octapeptide repeat (OR) region of PrP^C molecule deserves special mention because it is thought to play a special role in the maintenance of CNS myelin, although the results are sometimes conflicting. Mouse strains lacking or overexpressing the OR region show CNS myelin lesions [8,14,15], but the introduction of transgenes expressing OR-deleted PrPs in PrP^C KO mice restores their susceptibility to PrP^{SC} [16]. One Tg mouse strain expressing PrP with the insertion of additional OR regions does not show any CNS myelin lesions but only massive apoptosis of cerebellar granule cells [17,18]. However, it has been shown that the presence of redundant OR regions in the PrP molecule is causally related to some human prionopathies whose histopathological pictures include astrocytic proliferation and CNS spongiform vacuolation [19–23]. Furthermore, PrPs with supernumerary OR regions are toxic to cultured neuroblastoma cells [24]. Given that the OR region is the copper-binding region of the PrP^C molecule [3,25,26], this raises the unsolved question of the role of copper in CNS myelin maintenance.

PrP^Cs therefore have the fundamental task of maintaining myelins, but are not alone in doing this. For instance, PrP^Cs interact with glial fibrillary acidic protein (GFAP) [3,27,28], although the precise role of GFAP in maintaining the structure of CNS myelin remains elusive (reviewed in [29,30]). Increased GFAP protein levels are observed in the brains of PrP^{SC}-infected mice and coincide with astrocyte tumor necrosis factor (TNF)-α immunopositivity [31,32] (see also Section 2). For the link between PrP^C and Akt see Section 2.

More details concerning the relationships between PrP^Cs and myelin can be found in various reviews [1–4,7,27], but this review will mainly concentrate on the perspectives concerning the role of PrP^Cs in the pathogenesis of some human myelinopathies that do not belong to the classical human PrP-related diseases (here collectively called prionopathies) requiring conformational changes of PrP^Cs into pathological forms (e.g. PrP^{SC}) and their aggregation.

1.2. Cbl

In humans, acquired Cbl deficiency mainly affects the SC (subacute combined degeneration, SCD), PNS (polyneuropathy), and only rarely the brain [29,33,34]. However induced, acquired Cbl deficiency causes neuropathological and ultrastructural lesions (i.e. intramyelinic and interstitial edema, and glial activation) in the CNS and PNS (see also Section 2), and concomitant electrophysiological PNS abnormalities [33,34]. We have reproduced all of the morphological and ultrastructural hallmarks of human acquired Cbl deficiency-induced lesions in CNS and PNS of Cbl-deficient (Cbl-D) rats (reviewed in [29,33]), and provided evidence of electrophysiological damage in the PNS (reviewed in [29,33]).

Previous hypotheses concerning the pathogenesis of SCD have postulated a causal relationship between SCD and the impairment of both mammalian Cbl-dependent enzymes: *L*-methylmalonyl-coenzyme A (CoA) mutase (EC 5.4.99.2) requiring adenosyl-Cbl, and methionine synthase (EC 2.1.1.13) requiring methyl-Cbl, which leads to methylmalonic acid (MMA) and homocysteine (HCYS) accumulation in tissues (including CNS) and physiological fluids (including cerebrospinal fluid, CSF) (reviewed in [29,33,35]). We and other authors have previously discussed in detail the clinical and experimental findings in favor or against theories of a merely "biochemical" pathogenesis of SCD [29,35,36]. We have demonstrated that the severity of neuropathological features in the SC of Cbl-D rats does not correlate with the tissue or serum accumulation of MMA or HCYS [37]: there was no substantial increase in the severity of Cbl deficiency-induced lesions in SC white matter as the time of Cbl deficiency lengthened, thus making it unlikely that the SC or serum accumulation of MMA and/or HCYS is responsible for SC white matter lesions [37,38].

We have identified new pathogenetic mechanisms underlying SCD-like myelin lesions in the SC of adult Cbl-D rats by demonstrating that they are not caused by Cbl withdrawal alone but require concurrent abnormalities in the CNS synthesis and CSF levels of some myelin-related cytokines and growth factors (see Section 2.1.) [29,38]. These changes in the cytokine and growth factor network in rat Cbl-D CNS are etiologically related to Cbl-D status and, like the ultrastructural lesions in SC white matter, are substantially corrected by Cbl replacement [29,33]. In keeping with this, the SC white matter lesions in Cbl-D rats can also be "cured" by inactivating the local excess of TNF-α or nerve growth factor (NGF) by administering the specific antibodies (Abs) or anti-TNF-α (AT) transforming growth factor (TGF)-β, or, in the case of the local lacking molecule, by administering epidermal growth factor (EGF) or interleukin (IL)-6 [29,33]. Most of our findings concerning cytokine and/or growth factor regulation by Cbl in rat CNS have been confirmed in the CSF and/or serum of patients with SCD and/or pernicious anemia (PA): TNF-α levels are abnormally high and EGF levels abnormally low, and Cbl therapy normalizes serum levels [39]. These imbalances (including NGF increase) have been also confirmed in Cbl-D patients by others [40–42].

It has been suggested that the Cbl deficiency-induced decrease in CNS methionine synthase activity that leads to decreased methionine levels is a key factor in the pathogenesis of human SCD and SCD-like lesions in animal CNS (reviewed in [38]). However, CNS *S*-adenosyl-*L*-methionine (SAM) levels are decreased in SCD patients and patients with inborn errors of the methyl transferase pathway [43,44], unchanged in Cbl-D fruit bats [45], and increased in Cbl-D rats [46]. Furthermore, we have found that the level of the soluble (s) CD40:sCD40Ligand (L) dyad (which belongs to the TNF-α:TNF-α-receptor superfamily) is increased in the CSF but not the serum of Cbl-D rats at the time SC myelin lesions appear, and that treatment with Cbl, SAM, or TGF-β_1 normalizes or significantly reduces the CSF sCD40 levels and concomitantly restores normal SC myelin ultrastructure [47]. A substantial restoration of normal SC white matter myelin morphology and ultrastructure is obtained also by a treatment with anti-CD40 Abs [47]. These findings strongly suggest that the increase in CSF sCD40:sCD40L levels participates in the pathogenesis of SCD-like lesions in rat SC, even though SCD is not one of the neurological diseases with immunological and/or inflammatory characteristics, that are associated with CNS sCD40:sCD40L derangements (reviewed in [47]). Furthermore, it is worth remembering that SAM has well-known anti-TNF-α activity (reviewed in [47]), and so *in vivo* SAM treatment not only restores normal CNS methylations but also greatly reduces local sCD40:sCD40L levels: consequently, there is some contact