VITAMIN B_{12} AND NORMAL PRIONS: TWO FIELDS APPARENTLY SO FAR, BUT IN REALITY SO NEAR

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SUNTO. – La carenza cronica di cobalamina (Cbl) determina uno sbilanciamento di alcune citochine e fattori di crescita nel sistema nervoso centrale e periferico del ratto e nel siero e nel liquido cefalorachidiano di pazienti carenti in Cbl. Abbiamo recentemente ipotizzato che uno sbilanciamento dei livelli e/o della sintesi della proteina prionica normale (PrP^C) potrebbe essere coinvolto nella patogenesi della neuropatia da carenza di Cbl. Utilizzando dosaggi immunoenzimatici differenti e appropriati per le diverse molecole, abbiamo determinato i livelli di Cbl, tumour necrosis factor-α, epidermal growth factor, e PrP^C nel midollo spinale e nel liquido cefalorachidiano di ratti carenti in Cbl trattati o non trattati con alcune differenti molecole; nel siero, nel liquido cefalorachidiano di pazienti con carenza cronica di Cbl o sclerosi multipla; e in campioni post-mortem di midollo spinale prelevati da pazienti con sclerosi multipla e controlli. Abbiamo dimostrato che: (*i*) la carenza di Cbl determina un eccesso di PrP^C (soprattutto della regione octapeptide ripetuta (OR)) nel midollo spinale del ratto; (*ii*) l'aumento di PrP^C nel midollo spinale è mediato dall'aumento *in loco* di tumor necrosis factor-α mediato dalla carenza di Cbl e (*iii*) la concentrazione di PrP^C nel liquido cefalorachidiano e nel siero di pazienti carenti in Cbl sono significativamente aumentati rispetto ai controlli. I livelli di PrP^C nel liquido cefalorachidiano sono significativamente diminuiti nei pazienti con sclerosi multipla rispetto ai controlli. I livelli di Cbl, epidermal growth factor, and PrP^C sono significativamente diminuiti in campioni post-mortem di midollo spinale di pazienti con sclerosi multipla rispetto ai controlli.

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ABSTRACT. – Cobalamin (Cbl) deficiency causes an imbalance in some cytokines and growth factors in the central nervous system and peripheral nervous system of the rat, and in the serum and cerebrospinal fluid (CSF) of adult Cbl-deficient (Cbl-D) patients. We hypothesized that an imbalance in normal prion (PrP^C) levels and/or synthesis might be involved in the pathogenesis of Cbl-D neuropathy. Using different appropriate enzyme-linked immunosorbent assays (ELISAs), we determined the levels of Cbl, tumour necrosis factor-α, epidermal growth factor, and PrP^C in spinal cord (SC) and CSF of Cbl-D rats treated or not with different molecules; in serum, CSF from Cbl-D or multiple sclerosis (MS) patients; and in post-mortem SC samples taken from MS patients and control patients. We have demonstrated that: (*i*) Cbl deficiency induces excess PrP^C regions (particularly octapeptide repeated (OR) region) in rat SC; (*ii*) the SC increase is mediated by a local Cbl deficiency-induced excess of tumour necrosis factor-α; and (*iii*) CSF and serum PrP^C concentrations in Cbl-D patients are significantly higher than in controls. CSF PrP^C concentrations are significantly lower in MS patients than neurological controls. The Cbl, EGF, and PrP^C levels were significantly decreased in post-mortem MS SCs in comparison with controls.

1. INTRODUCTION

It is well-known that normal cellular prions (PrP^Cs) have a fundamental role in maintaining the structure and functions of normal central nervous system (CNS) myelin [1,reviewed in 2], although they are not alone in doing this, and their task is far from being fully elucidated. Important evidence of PrP^C role in CNS myelin maintenance comes from studies of PrP^C knock-out (KO) mice lacking one or more parts of the PrP^C molecule [1,reviewed in 2]. Furthermore, transgenic (Tg) mice expressing PrP^C point mutations, insertions or deletions develop a spectrum of neuropathological pictures reminiscent those of transmissible spongiform encephalopathies [1,reviewed in 2]. More in detail, mouse strains lacking or overexpressing the PrP^C octapeptide repeated (OR) region show CNS myelin lesion [1,reviewed in 2]. The presence of redundant OR regions in the PrP^C molecule is causally related to some human prionopathies whose histopathological pictures include CNS spongiform vacuolation and astrocytic proliferation. All of the above studies highlighted that CNS myelin lesions may also be caused merely by local quantitative PrP^C abnormalities [1,reviewed in 2].

We demonstrated that the severity of lesions in the spinal cord (SC) white matter of cobalamin-deficient (Cbl-D) rats does not correlate with the accumulation of methilmalonic acid and homocysteine (*i.e.* the

two metabolites which accumulate when Cbl is lacking) in their SCs and sera, because no substantial increase in the severity of Cbl deficiency-induced lesions in the SC white matter was observed as methilmalonic acid and homocysteine accumulated in the SC [3]. Thereafter, we have identified new pathogenetic mechanisms of the myelin lesion of central Cbl-D neuropathy by demonstrating that the SC myelin lesions are caused not by mere Cbl withdrawal but by the Cbl deficiency-induced abnormalities in the SC and cerebrospinal fluid (CSF) of some myelin-related cytokines and growth factors [3]. Briefly, local tumour necrosis factor(TNF)-α levels and/or synthesis are abnormally high and local epidermal growth factor (EGF) levels and/or synthesis abnormally low in SC of Cbl-D rats [3]. In other words, TNF-α excess becomes myelinotoxic and simultaneous EGF lack deprives CNS of its myelintrophic action. Most of our findings concerning cytokine and growth factor derangements in CNS of Cbl-D rats were confirmed by us in human CSF of patients with Cbl-D neuropathy (subacute combined degeneration). The Cbl replacement treatment substantially corrected the above abnormalities in Cbl-D patients and Cbl-D rats [3].

On the basis of all the above, we posited the working hypothesis that there may be a link between Cbl and PrP^Cs and that this link is deranged in Cbl-D neuropathy because of the Cbl deficiency-induced imbalance in CNS cytokine and/or growth factor network. In particular, it should be emphasized that: *i*) PrP^C synthesis has been shown to be regulated *in vitro* by TNF-α and EGF; *ii*) TNF-α levels are markedly increased in the brain of scrapie-infected mice; and *iii*) myelin vacuolation, reactive astrocytosis, and microglial activation are neuropathological features common to Cbl-D central neuropathy and the CNS of most prionopathies [reviewed in 2].

2. Materials and Methods

2.1. In vivo *experiments*

We used non-inbred adult male albino rats (Sprague–Dawley strain) (Charles River Italia, Calco, Italy). The experimental groups consisted of: controls, laparotomized (LPT) rats, and Cbl-D rats (totally gastrectomized rats or rats on a Cbl-D diet). Some of these rats received different drugs by means of intracerebroventricular (i.c.v.) microinjections. CSF and SC samples were collected as previously described [2].

2.2. Patients

Patients with pernicious anaemia, amyotrophic lateral sclerosis or multiple sclerosis (MS), and controls were recruited from different hospital centers. Venous blood and CSF samples were collected as previously described [4]. The post-mortem SC samples from control and MS patients were supplied by the U.K. Multiple Sclerosis Tissue Bank, London [5].

2.3. Enzyme-linked immunosorbent assays (ELISA)

An home-made ELISA was used to determine PrP^C concentration [2,4]. CSF and SC TNF-α levels were determined using an ELISA kit (Rat TNF-α, Biosource, Carlsbad, CA; detection limit: < 4 pg/ml) [2].

3. Results

SC PrP^C levels had increased by the time local myelin lesions appeared. This increase was mediated by excess myelinotoxic TNF-α and prevented by repeated i.c.v. injections of EGF, which proved to be as effective as Cbl in preventing Cbl deficiency-induced SC myelin lesions [2]. Repeated i.c.v. injections of anti-OR region antibodies prevented the Cbl deficiency-induced lesions of SC myelin [2]. *In vivo* Cbl or EGF treatment significantly increased SC PrP^C mRNA levels in Cbl-D rats [2].

We have always considered our studies of equivalent animal models of acquired Cbl-D neuropathy as prolegomena to our studies of adult patients with clinically confirmed severe Cbl deficiency. We demonstrated that: *i*) CSF PrP^C levels were significantly higher in the therapy-free Cbl-D patients (*i.e.* with subacute combined degeneration) than in neurological controls [4]; *ii*) CSF PrP^C levels correlated significantly with CSF Cbl levels [4], and *iii*) CSF PrP^C levels were normal in the patients with amyotrophic lateral sclerosis [4] or Alzheimer's disease [6]. Instead, we found significantly decreased CSF PrP^C levels in MS patients (regardless of its clinical course) [4]. The PrP^C levels of post-mortem SC samples of MS patients were also decreased [5]. The decreased PrP^C availability in MS SC surely represents one of the causes of the remyelination failure in MS.

4. Conclusions

We were the first to demonstrate that an experimental myelinolytic neuropathy (Cbl-D central neuropathy) is also caused by a local excess of PrP^Cs [2,7,8] that do not show any conformational change like in the case of scrapie PrPs. Conversely, PrP^C levels are very low in CNS of a typical demyelinating disease, MS [4]. It should be also noted that SC is the CNS part most severely affected by Cbl deficiency and in MS. In other words, our findings are in agreement with the results of PrP^C KO-or PrP^C Tg-mice showing that any deregulation (excess or deficiency) in CNS PrP^C levels jeopardizes CNS normal myelin maintenance [reviewed in 7,8]. Finally, we demonstrated for the first time that the Cbl and EGF buffering of SC PrP^C levels is crucial for keeping SC myelin normal, because Cbl and EGF protect SC myelin against the myelin-damaging excess or lowering of SC PrP^C levels [7,8].

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